# ISOLATION AND IDENTIFICATION OF NON FERMENTOR GNB AND DETECTION OF *bla* VIM MEDIATED CARBAPENAMASE RESISTANCE FROM CLINICAL ISOLATES IN A TERTIARY CARE HOSPITAL

**DISSERTATION SUBMITTED TO**

**In partial fulfillment of the requirement for the degree of**

**DOCTOR OF MEDICINE IN MICROBIOLOGY**

**(Branch IV) M. D. (MICROBIOLOGY)**

**of**

**THE TAMIL NADU DR. M. G. R MEDICAL UNIVERSITY**

**CHENNAI- 600032**


**DEPARTMENT OF MICROBIOLOGY**

**TIRUNELVELI MEDICAL COLLEGE**

**TIRUNELVELI- 11**

**MAY 2018**

# BONAFIDE CERTIFICATE

This is to certify that the dissertation entitled **"ISOLATION AND IDENTIFICATION OF NON FERMENTOR GNB AND DETECTION OF *bla* VIM MEDIATED CARBAPENAMASE RESISTANCE FROM CLINICAL ISOLATES IN A TERTIARY CARE HOSPITAL"** submitted by **Dr. R.P.R. Suyambu Meenakshi** to the Tamilnadu Dr. M.G.R Medical University, Chennai, in partial fulfillment of the requirement for the award of M.D. Degree Branch – IV (Microbiology) is a bonafide research work carried out by her under direct supervision & guidance.

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## CERTIFICATE

This is to certify that the Dissertation **“ISOLATION AND IDENTIFICATION OF NON FERMENTOR GNB AND DETECTION OF *bla* VIM MEDIATED CARBAPENAMASE RESISTANCE FROM CLINICAL ISOLATES IN A TERTIARY CARE HOSPITAL”** presented herein by **Dr. R.P.R. Suyambu Meenakshi** is an original work done in theDepartment of Microbiology, Tirunelveli Medical College Hospital,Tirunelveli for the award of Degree of M.D. (Branch IV) Microbiologyunder my guidance and supervision during the academic period of 2015 -2018.

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## DECLARATION

I solemnly declare that the dissertation titled**"ISOLATION AND IDENTIFICATION OF NON FERMENTOR GNB AND DETECTION OF *bla* VIM MEDIATED CARBAPENAMASE RESISTANCE FROM CLINICAL ISOLATES IN A TERTIARY CARE HOSPITAL"** is done by me at Department of Microbiology,Tirunelveli Medical College hospital,Tirunelveli. I also declare that this bonafide work or a part of this work was not submitted by me or any others for any award, degree, or diploma to any other University, Board, either in India or abroad.

The dissertation is submitted to The Tamilnadu Dr. M.G.R.MedicalUniversity towards the partial fulfilment of requirements for the award ofM.D. Degree (Branch IV) in Microbiology.

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## LIST OF ABBREVATIONS

| | |
|---|---|
| NFGNB | Non fermenter Gram negative bacilli |
| *P.aeruginosa* | *Pseudomonas aeruginosa* |
| *P.putida* | *Pseudomonas putida* |
| *A.baumani* | *Acinetobacter baumani* |
| MBL | Metallobetalactamase |
| Opr | Outer membrane porin |
| CLSI | Clinical Laboratory Standards Institute |
| CDT | Combined Disk Synergy Test |
| DDST | Double Disk Synergy Test |
| EDTA | Ethylene Diamine Tetra Acetic Acid |
| E test | Epsilometer test |
| MIC | Minimum Inhibitory Concentration |
| MEM | Meropenem |
| MP | Meropenem |
| MPI | Meropenem with EDTA |
| CFU | Colony Forming Unit |
| MHA | Muller Hinton Agar |

| | |
|---|---|
| ATCC | American Type Culture Collection |
| CRNFGNB | carbapenem resistant Non frementer Gram negative bacilli |
| CSNFGNB | carbapenem sensitive Non frementer Gram negative bacilli |
| NDM | NewDelhimetallobetalactamase |
| IMP | Imipenemases |
| VIM | Verona integrin encoded metallo β lactamase |
| SPM | Sao Paulo metallo β lactamase |
| GIM | German imipenemase |
| SIM | Seoul imipenemase |
| *bla* | Betalactamase |
| µg | microgram |
| PCR | Polymerase chain reaction |
| DNA | Deoxy Ribonucleic Acid |
| IC | Internal Control |
| CT | Cross Threshold |

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PROTOCOL TITLE: ISOLATION AND IDENTIFICATION OF NON FERMENTOR GNB AND DETECTION OF BLA VIM MEDIATED CARBAPENAMASE RESISTANCE FROM CLINICAL ISOLATES IN A TERIATARY CARE HOSPITAL

PRINCIPAL INVESTIGATOR: Dr. SUYAMBU MEENAKSHI.R.P.R. MBBS.,
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*Dear , Dr. Suyambu Meenakshi, R.P.R., MBBS.,, The Tirunelveli Medical College Institutional Ethics Committee (TIREC) reviewed and discussed your application during the IEC meeting held on 05.08.2016.*

**THE FOLLOWING DOCUMENTS WERE REVIEWED AND APPROVED**

1. TIREC Application Form ☐
2. Study Protocol ☐
3. Department Research Committee Approval ☐
4. Patient Information Document and Consent Form in English and Vernacular Language ☐
5. Investigator's Brochure ☐
6. Proposed Methods for Patient Accrual Proposed ☐
7. Curriculum Vitae of the Principal Investigator ☐
8. Insurance /Compensation Policy ☐
9. Investigator's Agreement with Sponser ☐
10. Investigator's Undertaking ☐
11. DCGI/DGFT approval ☐
12. Clinical Trial Agreement (CTA) ☐
13. Memorandum of Understanding (MOU)/Material Transfer Agreement (MTA) ☐
14. Clinical Trials Registry-India (CTRI) Registration ☐

**THE PROTOCOL IS APPROVED IN ITS PRESENTED FORM ON THE FOLLOWING CONDITIONS**

1. The approval is valid for a period of 2 year/s or duration of project whichever is later
2. The date of commencement of study should be informed
3. A written request should be submitted 3weeks before for renewal / extension of the validity
4. An annual status report should be submitted.
5. The TIREC will monitor the study
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7. The PI should report to TIREC within 7 days of the occurrence of the SAE. If the SAE is Death, the Bioethics Cell should receive the SAE reporting form within 24 hours of the occurrence.
8. In the events of any protocol amendments, TIREC must be informed and the amendments should be highlighted in clear terms as follows:
   a. The exact alteration/amendment should be specified and indicated where the amendment occurred in the original project. (Page no. Clause no. etc.)
   b. The PI must comment how proposed amendment will affect the ongoing trial. Alteration in the budgetary status, staff requirement should be clearly indicated and the revised budget form should be submitted.
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**STANDS APPROVED UNDER SEAL**

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## CERTIFICATE - II

This is certify that this dissertation work title **"ISOLATION AND IDENTIFICATION OF NON FERMENTOR GNB AND DETECTION OF bla VIM MEDIATED CARBAPENAMASE RESISTANCE FROM CLINICAL ISOLATES IN A TERTIARY CARE HOSPITAL"** of the candidate **Dr. R.P.R. Suyambu Meenakshi** with registration Number **201514304** for the award of **M.D.** Degree in the branch of **MICROBIOLOGY (IV).** I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains from introduction to conclusion page and result shows **0 percentage** of plagiarism in the dissertation.

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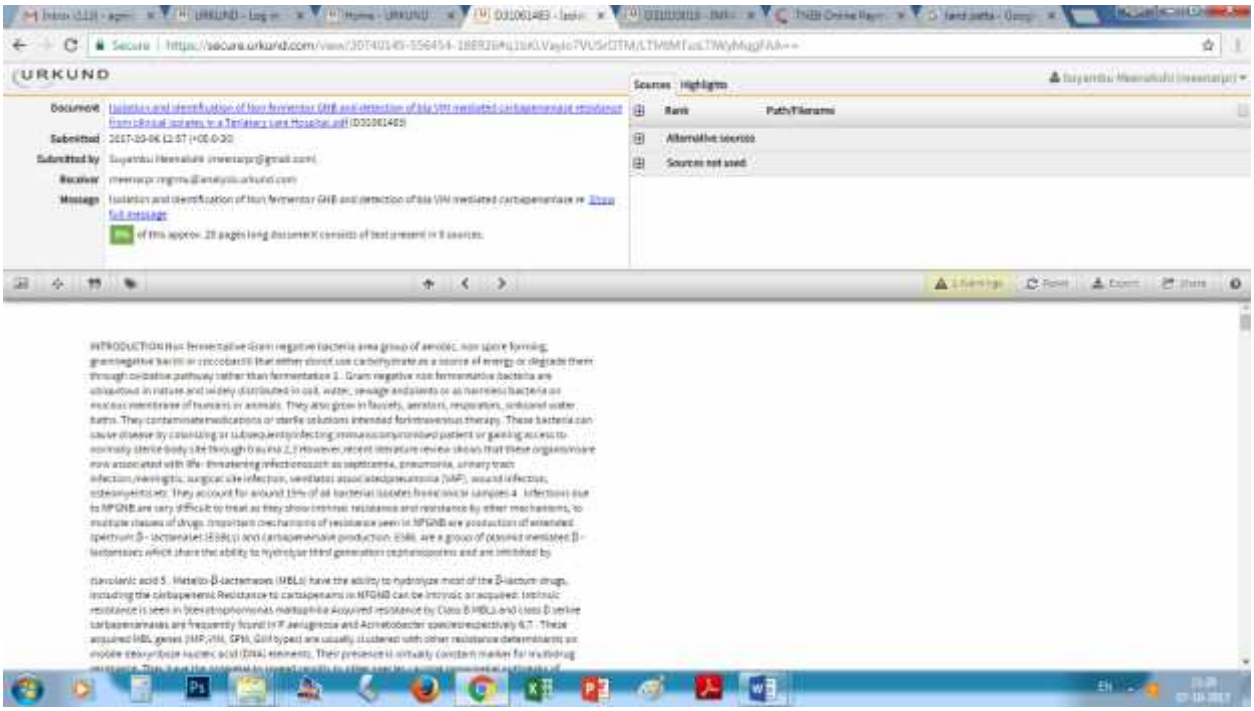
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INTRODUCTION Non fermentative Gram negative bacteria are a group of aerobic, non spore forming, gramnegative bacilli or coccobacilli that either donot use carbohydrate as a source of energy or degrade them through oxidative pathway rather than fermentation 1. Gram negative non fermentative bacteria are ubiquitous in nature and widely distributed in soil, water, sewage andplants or as harmless bacteria on mucous membrane of humans or animals. They also grow in faucets, aerators, respirators, onboard water baths. They contaminatemedications or sterile solutions intended forintravenous therapy. These bacteria can cause disease by colonizing or subsequentlyinfecting immunocompromised patient or gaining access to normally sterile body site through trauma 2,3 However,recent literature review shows that these organismsare now associated with life- threatening infectionssuch as septicemia, pneumonia, urinary tract infection,meningitis, surgical site infection, ventilator associatedpneumonia (VAP), wound infection, osteomyelitis etc. They account for around 15% of all bacterial isolates fromclinical samples4. Infections due to NFGNB are very difficult to treat as they show intrinsic resistance and resistance by other mechanisms, to multiple classes of drugs. Important mechanisms of resistance seen in NFGNB are production of extended spectrum β - lactamases(ESBLs) and carbapenemase production. ESBL are a group of plasmid mediated β - lactamases which share the ability to hydrolyse third generation cephalosporins and are inhibited by
clavulanic acid 5. Metallo-β-lactamases (MBLs) have the ability to hydrolyze most of the β-lactam drugs, including the carbapenems.Resistance to carbapenems in NFGNB can be intrinsic or acquired. Intrinsic resistance is seen in Stenotrophomonas maltophilia.Acquired resistance by Class B MBLs and class D serine carbapenemases are frequently found in P.aeruginosa and Acinetobacter speciesrespectively 6,7. These acquired MBL genes (IMP,VIM, SPM, GIM types) are usually clustered with other resistance determinants on mobile deoxyribose nucleic acid (DNA) elements. Their presence is virtually constant marker for multidrug

# 1. INTRODUCTION

Non fermentative Gram negative bacteria are a group of aerobic, non spore forming, gram negative bacilli or coccobacilli that either do not use carbohydrate as a source of energy or degrade them through oxidative pathway rather than fermentation[1].

Gram negative non fermentative bacteria are ubiquitous in nature and widely distributed in soil, water, sewage and plants or as harmless bacteria on mucous membrane of humans or animals. They also grow in faucets, aerators, respirators, sinks and water baths. They contaminate medications or sterile solutions intended for intravenous therapy. These bacteria can cause disease by colonizing or subsequently infecting immunocompromised patient or gaining access to normally sterile body site through trauma[2,3]

However, recent literature review shows that these organisms are now associated with life-threatening infections such as septicemia, pneumonia, urinary tract infection, meningitis, surgical site infection, ventilator associated pneumonia (VAP), wound infection, osteomyelitis etc. They account for around 15% of all bacterial isolates from clinical samples[4].

Infections due to NFGNB are very difficult to treat as they show intrinsic resistance and resistance by other mechanisms, to multiple classes of drugs. Important mechanisms of resistance seen in NFGNB are production of extended spectrum β- lactamases (ESBLs) and carbapenemase production. ESBL are a group of plasmid mediated β-lactamases which share the ability to hydrolyse third generation cephalosporins and are inhibited by

clavulanic acid[5]. Metallo-β-lactamases (MBLs) have the ability to hydrolyze most of the β-lactum drugs, including the carbapenems

Resistance to carbapenems in NFGNB can be intrinsic or acquired. Intrinsic resistance is seen in *Stenotrophomonas maltophilia.* Acquired resistance by Class B MBLs and class D serine carbapenamases are frequently found in *P.aeruginosa* and *Acinetobacter* species respectively[6,7]. These acquired MBL genes (IMP,VIM, SPM, GIM types) are usually clustered with other resistance determinants on mobile deoxyribose nucleic acid (DNA) elements. Their presence is virtually constant marker for multidrug resistance. They have the potential to spread rapidly to other species causing nosocomial outbreaks of carbapenem resistance. Hence, regular monitoring of carbapenem resistance is essential in developing strategies to control infection.

## 2. AIMS AND OBJECTIVES

- To isolate and identify Non fermentor Gram negative bacilli from clinical specimens.
- To determine the Antibiogram of identified non fermenting gram negative bacilli .
- To detect the production of carbapenemase by non fermenting gram negative bacilli using phenotypic methods.
- To detect the presence of metallo betalactamse using *bla* VIM gene by Real time PCR

# 3. REVIEW OF LITERATURE

Non fermenting Gram negative bacilli are a group of taxonomically diverse organisms that have the common feature of not utilizing glucose or use it by pathways other than fermentation, failing to acidify the butt of Triple Sugar Iron(TSI) agar [1,2].

In order to gain a clear understanding of NFGNB, it is important to understand the different metabolic pathways utilized by these organisms.

**Bacterial metabolism[1,7,8]**

The bacterial degradation of carbohydrates proceeds by several metabolic pathways in which hydrogen ions are successively transferred to compounds of higher redox potential, with the ultimate release of energy in the form of adenosine triphosphate (ATP).

All six-, five-, and four- carbon carbohydrates are initially degraded to pyruvic acid, an initial intermediate. Glucose is the main carbohydrate source of the carbon for bacteria and degradation proceeds by three major pathways (Rudolph Hugh):

1. The Embden- Meyerhof- Parnas pathway

2. The Entner- Doudoroff pathway

3. The Warburg-Dickins/ The Hexose monophosphate pathway

Glucose is converted to pyruvic acid by a series of reactions in each of these three pathways. Bacteria use one or more of these pathways of glucose metabolism depending on the availability of oxygen and the presence of enzymes within the organism.

**The Embden- Meyerhof- Parnas (EMP) pathway**

It is also called the glycolytic or anaerobic pathway because glucose is degraded without the use of oxygen. This has been called the fermentative pathway as so organic compounds serve as the final electron acceptors. End products formed from this pathway are the mixed acids, which account for the drop in the pH in the fermentation tests.

**The Entner – Doudoroff (ED) Pathway**

This is called the aerobic pathway because oxygen is required for glycolysis. This has been classically called the oxidative pathway, because pyruvic acid transfers the electrons into Kreb's cycle, where they are linked to elemental oxygen to form water. Therefore, through this pathway, the carbohydrates are oxidatively metabolized, where oxygen is required as the terminal electron acceptor. The acids formed in this pathway are extremely weak and hence cannot be detected by the routinely used tests. Many of NFGNB use this pathway of carbohydrate metabolism.

**The Warburg – Dickens/ Hexose Monophosphate Pathway**.

This pathway is a hybrid between the EMP and ED pathways. It is used mainly by facultatively anaerobic organisms. Mixed acids are formed as end products in this pathway also. Hence the organisms using this pathway give fermentative reaction in routinely used biochemical tests.

**3.1 Habitat**

Nonfermentative, gram-negative bacilli are found in most environments, in soil and water, on plants and decaying vegetation, and in many foodstuffs. They prefer moist

environments, and in hospitals they can be isolated from nebulizers, dialysate fluids, saline, catheters and other devices.

Basically, they are saprophytes and previously they were considered as contaminants or commensals of little significance.[9,10] However, recent literature review shows that these organisms are now associated with life-threatening infections such as septicemia, pneumonia, urinary tract infection, meningitis, surgical site infection, ventilator associated pneumonia (VAP), wound infection, osteomyelitis etc.[10]

They account for around 15% of all bacterial isolates from clinical samples.[11] Routinely they are identified only in few laboratories in India as they are slow growing and require special culture media and biochemical tests for their identification.[9]*Pseudomonas* and *Acinetobacter* species together accounted for 84.06% of NFGNB isolates[21,22]

A prospective study carried out at the Department of Microbiology, Government Medical College Jammu (J&K) showed the isolation rate of NFGNB was 13.5%[12]

Another prospective study done by Benachinmardi, *et al* conducted in the Department of Microbiology at a tertiary care teaching hospital over a period of 2 months from September to October 2013 had the isolation rate of 3.58%[13]which is in parallel to studies by Malini *et al*[14]. and Bruno *et al*[15].whose isolation rate was 4.5% and 2.18% respectively. On the other hand, Samanta *et al*[16]. Eltahawy and Khalaf[17]Vij aya *et al.*[18] and Sidhu *et al*[19]. have reported higher rateof isolation i.e., 10%, 16%, 21.80% and 45.9% respectively

**Clues to identify nonfermenter [1]**

1. Lack of evidence for glucose fermentation

2. Positive cytochrome oxidase reaction

3. Failure to grow on Mac.Conkey agar

4. Thin, gram-negative bacilli or coccobacilli on Gram stain

5. No acid production in the slant or butt of TSIA or KIA

6. Resistance to a variety of classes of antimicrobial agents.

**3.2 Taxonomy**

The NFGNB do not fit into a single family of well- characterized genera[1].The correct taxonomic placement of many of the NFGNB is still unsolved.

The medically important non-fermenters can also be classified on the basis of the presence or absence of motility and the type of flagella present on the strains that are motile as follows[1]

**Classification of NFGNB based on motility and type of flagella**

**MOTILE WITH POLAR FLAGELLA**

**Family *Pseudomonadaceae***

(rRNA group I)

Genus *Pseudomonas*

**Family *Burkholderiaceae***

(rRNA group II)

Genus *Burkholderia*

Genus *Cupriavidus*

Genus *Lautropia*

Genus *Pandorea*

Genus *Ralstonia*

**Family *Comamonadaceae***

(rRNA group III)

Genus *Comamonas*

Genus *Acidovorax*

Genus *Delftia*

**Family *Caulobacteriaceae***

(rRNA group IV)

Genus *Brevundimonas*

**Family *Xanthomonadaceae***

(rRNA Group V)

Genus *Stenotrophomonas*

**Family *Sphingomonadaceae***

Genus *Sphingomonas*

**Family *Oceanospirillaceae***

Genus *Balneatrix*

**Family *Alteromonadaceae***

Genus *Shewanella*

Genus *Alishewanella*

**Family *Oxalobacteriaceae***

Genus *Herbaspirillum*

Genus *Massilia*

**Family *Methylobacteriaceae***

Genus *Methylobacterium*

Genus *Roseomonas*

Organisms with uncertain taxomonic

positions

CDC Groups Ic, O-1, O-2, O-3, Vb- 3

**MOTILE WITH PERITRICHOUS FLAGELLA**

**Family *Alcaligenaceae***

Genus *Achromobacter*

Genus *Alcaligenes*

Genus *Bordetella*

Genus *Kerstersia*

Genus *Oligella*

**Family *Rhizobaceae***

Genus *Rhizobium*

**Family *Brucellaceae***

Genus *Ochrobactrum*

**Family *Halomonadaceae***

Genus *Halomonas*

**NONMOTILE OXIDASE POSITIVE**

**Family *Flavobacteriaceae***

Genus *Flavobacterium*

Genus *Bergeyella*

Genus *Chryseobacterium*

Genus *Empedobacter*

Genus *Myroides*

Genus *Weeksella*

**Family *Sphingobacteriaceae***

Genus *Sphingobacterium*

Genus *Pedobacter*

**Family *Moraxellaceae***

Genus *Moraxella*

Genus *Psychrobacter*

**Family *Neisseriaceae***

Genus *Neisseria*

**Family *Alcaligenaceae***

Genus Oligella

**Family *Rhodobacteraceae***

Genus *Paracoccus* (EO-2)

Organisms with uncertain

taxonomic positions

CDC groups EO-3, EO-4, EF-4b

CDC groups IIc, IIe, IIg, IIh, IIi

Gilardi rod group 1

**NONMOTILE OXIDASE NEGATIVE**

**Family *Moraxellaceae***

Genus *Acinetobacter*

**Family *Alcaligenaceae***

Genus *Bordetella*

Organisms with uncertain taxonomic positions

CDC group NO-1

CDC group EO- 5

## 3.3 DESCRIPTION OF IMPORTANT GROUPS:

### 3.3.1. Pseudomonads

This consists of the genus Pseudomonas and some closely related genera like *Burkholderia, Comamonas, Acidovorax, Hydrogenophaga, Brevundimonas, Stenotrophomonas* and *Xanthomonas* [1,2].

**Family *Pseudomonadaceae***

This consists of the genus *Pseudomonas*, which is the best known nonfermenter. Presently, there are 160 species of *Pseudomonas*, of which 12 are medically important.

To aid in identification, the species are divided into the following important groups

**Flourescent group:**

The species within the group produces water soluble pyoverdin pigment that fluoresces white to blue green pigment under long wave length (400nm) ultraviolet light.

The species included in this group are

- *Pseudomonas aeruginosa*
- *Pseudomonas fluorescens*
- *Pseudomonas putida*

***Pseudomonas aeruginosa***

*Pseudomonas aeruginosa* first documented as the source of 'blue pus' in wounds and hence named Bacillus pyocyaneus by Gessard. The name was altered by S edillot in to pseudomonas *pyocyanea* in 1850.

Pseudomonas are rod-shaped, gram-negative bacteria, motile by means of polar flagella. They are not acid-fast and do not form spores, prosthecae, or cysts. They have an absolute aerobic metabolism and the catalase reaction is positive

Most strains of *P. aeruginosa* will also produce the blue, water-soluble pigment pyocyanin. No other nonfermentative,gram-negative bacillus produces pyocyanin. About 4% of clinical strains of *P. aeruginosa*, however, do not produce pyocyanin. They also produce pyoverdin, a yellow-green or yellow-brown pigment. Pyoverdin is water-soluble and fluoresces under short-wavelength ultraviolet light. Pyorubin [red] and pyomelanin [brown or black],are occasionally produced by strains of *P. aeruginosa*.

**Virulence Factors**: *Pseudomonas aeruginosa* can producea variety of factors that lead to its pathogenicity, such as endotoxin(lipopolysaccharide [LPS]), motility, pili,

capsule, and several exotoxins—proteases, hemolysins, lecithinase, elastase,and DNase[25,26]. The most important exotoxin is exotoxin A; this exotoxin functions similarly to diphtheria toxin by blocking protein synthesis.

In lower respiratory tract infections of patients with Cystic fibrosis, most *P. aeruginosa* strains produce mucoid colonies caused by the overproduction of alginate, a polysaccharide polymer. The production of mucoid colonies in strains isolated from patients with CF can be a helpful identification.

. *P. aeruginosa* account for 5% to15% of all nosocomial infections, especially pneumonia and bacteremia. .*P. aeruginosa* is the leading cause of nosocomial respiratory tract infections. A large variety of clinical diseases have been documented as being caused by *P. aeruginosa*, including bacteremia, often presenting with ecthyma gangrenosum of the skin, wound infections, pulmonary disease, especially among individuals with CF, nosocomial urinary tract infections (UTIs),endocarditis, infections following burns or trauma and, in rar ecases, central nervous system infections, including meningitis.

When *P. aeruginosa* is isolated froma sterile body site such as blood, pleural fluid, joint fluids or tissues, or cerebrospinal fluid (CSF), it almost always constitutes a true infection. *P. aeruginosa* can colonize mucosal surfaces such as the oropharynx. Patients in the intensive care unit (ICU) who are mechanically ventilated may quickly become colonized with *P. aeruginosa*.

**Diagnostic criteria:**

- Slender Gram negative rod
- Motile

- Catalase positive
- Oxidase positive
- β-hemolytis on sheep blood agar (SBA) and will produce flat spreading colonies with a characteristic metallic sheen.
- Fruity,grapelike odor caused by the presence of 2-aminoacetophenone.
- Denitrification of nitrates and nitrites,
- Arginine dihydrolase (ADH)–positive,
- Growth at 42° C – positive
- Citrate utilization –positive
- Acetamide utilization- positive

**Treatment**

*P. aeruginosa* is innately resistant to penicillin, ampicillin, first- and second-generation cephalosporins, trimethoprimsulfamethoxazole (SXT), chloramphenicol, and tetracyclines. Itis usually susceptible to the aminoglycosides, semisynthetic penicillins such as piperacillin and ticarcillin, third- and fourth-generation cephalosporins (ceftazidime and cefepime, respectively), and carbapenems and the fluoroquinolones.

***Pseudomonas fluorescens*** **and** ***Pseudomonas putida*** do not possess distinctive colony morphology or odour [2]. They are present in water and soil and may sometimes be found contaminating the water source in hospitals. These species have very rarely been associated with human infections. *P. fluorescens* .Pseudobacteremia is usually seen due to contamination of the catheters. [1,2,27]

**Diagnostic criteria:**

- Inability to reduce nitrates to nitrogen gas
- Production of acid from xylose .

*P. fluorescens* can be able to grow at 4°C and has the ability to hydrolyze gelatin; *P. putida* can do neither.

***Pseudomonas* Nonfluorescent Group**

***Pseudomonas stutzeri***

*Pseudomonas stutzeri* is a rare isolate and even rarer pathogen in the clinical laboratory. In the immunocompromised host, *P. stutzeri* has been reported to be responsible for diseases that include septicemia, meningitis in the HIV infected patient, pneumonia (especially in CF and other immunocompromised patients), endocarditis, postsurgical wound infections, septic arthritis, conjunctivitis, and UTIs.

*P.stutzeri* is a soil denitrifier and can grow in an anaerobic environment in nitrate-containing media, producing nitrogen gas.

**Diagnostic criteria**

- Produces wrinkled, leathery, adherent colonies that may produce a light-yellow or brown pigment .
- Arginine dihydrolase negative
- Starch hydolysis positive.

These features distuiguishes it from *Burkholderia pseudomallei.*

***Pseudomonas mendocina***

*Pseudomonas mendocina* can be found in soil and water but is rarely isolated from human specimens.

**Diagnostic criteria**

- Motile by means of a single polar flagellum
- Oxidizes glucose and xylose
- Does not hydrolyze starch
- Produces non wrinkled, flat colonies that may appear with a yellowish-brown pigment.
- Oxidase positive
- ADH positive like *P. aeruginosa*,
- Does not produce pyoverdin
- Acetamide - negative.

**Alcaligenes group**

The organisms in this group are *P. alcaligenes*, *P. pseudoalcaligenes* and CDC group 1. These are also very rare human pathogens[28]. They are characterized by being asacchrolyitc or only weakly sacchrolytic in OF glucose medium. There have been reports of *P.alcaligenes* causing eye infections, empyema and endocarditis.[29,30]

***Pseudomonas luteola* and *Pseudomonas oryzihabitans***

Both the organisms in this group are rarely isolated from clinical samples. *P. luteola* has been known to cause infections like bacteremia, endocarditis, mengitis, osteomyelitis and peritonitis[31,32,33].

*P.oryzihabitans* is associated with intravascular catheter related infections in immunocompromised patients.[34]

**Diagnostic criteria**

- Oxidase negative
- Catalase positive
- Motile
- Production of intracellular non diffusible yellow pigment.
- Produce wrinkled or rough colonies at 48 hours .

*P. luteola* can be differentiated from *P. oryzihabitans* by the *ortho*-nitrophenyl-galactopyranoside (ONPG) test and esculin hydrolysis.

### 3.3.2.Genus *Acinetobacter*

The genus *Acinetobacter is a* member of the family *Moraxellacea*e. It consists of 25 DNA homology groups or genomospecies. Only 11 species have been officially named[35].

The important species belonging to this genus are *Acinetobacter baumannii*, *Acinetobacter lwoffii*, *Acinetobacter haemolyticus, Acinetobacter calcoaceticus* and *Acinetobacter junii*[1,2].

*Acinetobacter* spp. are ubiquitous in the environment in soil, water, and foodstuffs. In the hospital environment, they have been associated with ventilators, humidifiers, catheters, and other devices. About 25% of adults carry the organisms on their skin, and

about 7% carry the organism in their pharynx. If not already harboring *Acinetobacter* spp., hospitalized patients become easily colonized.

In a study in Tehran, from 100 collected samples in ICU of Rasoul Akram hospital, 21 samples (21%) were *A. baumannii*[36]. In another study of Rit and his colleagues in 2012 among 4180 clinical isolates 74.02% *A.baumannii* and 25.98% other types of Acinetobacter have been diagnosed[37].

Diseases with which they have been associated (in particular *A. baumanii*) include UTIs, pneumonia, ventilator associated pneumonia ,tracheobronchitis, or both, endocarditis, with up to a 22% mortality, septicemia, meningitis, often as a complication of intrathecal chemotherapy for cancer, and cellulitis, usually as a result of contaminated indwelling catheters, trauma, burns, or introduction of a foreign body[38,39,40].

All the *Acinetobacter* spp. are strictly aerobic, and they appear as gram-negative coccobacilli or even gram-negative cocci on Gram stain. *Acinetobacter* organisms can appear as gram-positive cocci in smears made from blood culture bottles[41].

**Diagnostic criteria**

- Oxidase negative
- Catalase positive
- Nonmotile.
- Colonies on blood agar appear small to medium sized, smooth, translucent to opaque with entire edges.
- Most of the strains grow well on MacConkey agar and produce pale or slightly pinkish colonies.

- Penicillin resistance

*Acinetobacter baumannii* is the species most commonly isolated from the clinical specimens[42]. It is sacchrolytic and acidifies most of the OF test carbohydrates. Rapid identification is made by the ability of *A. baumannii* in fermenting 1% and 10% lactose.

Isolates of *A. baumannii* are often resistant to many antimicrobials, including penicillins, first- and second-generation cephalosporins, and fluoroquinolones. Many strains exhibit resistance to carbapenems; carbapenemases have been reported throughout the United States. These isolates have been referred to as CRAB, or carbapenem-resistant *A. baumanii*. CRAB isolates are usually only susceptible to colistin and tigecycline.

Feizabadi *et al.* reported the prevalence of susceptibility of *A. baumannii* to imipenem, meropenem, piperacillin ‑tazobactam, and amikacin rate of 50.7%, 50%, 42.1%, and 38.2%, respectively[43].

*Acinetobacter johnsonii, A.lwoffi* and *A.radioresistens* are commensals of the human skin as well as oropharynx and vagina. *A.lwoffi*has been associated with meningitis[44]. *A.junii*is a rare cause of ocular infections and bacteremia , particularly in paediatric patients[45]. Other rarely isolated species like *A.ursingii* and *A.schindleri* have been recovered from clinical samples. *A.ursingii* has been shown to cause bloodstream infections in hospitalized patients[46]. *A schindleri* is regarded as a contaminant with no clinical significance.

### 3.3.3. Genus *Burkholderia*

All are aerobic, gram negative, non sporing, intra cellular bacteria. They are inhabitants of environment,transmitted to humans with heavily contaminated medical devices[1].

They are similar to *Pseudomonas;* however, they differ from the later in being

- Bipolar stained (safety pin appearance)
- Resistance to polymyxin B

The genus contains

*B.pseudomallei*

*B.mallei*

*B.gladioli*

***Burkholderia pseudomallei***

*B. pseudomallei* can be used as a potential agent of biological warfare. *B. pseudomallei i*s perhaps the most virulent among the non-fermenters. Several virulence factors am described such as polysaccharide capsule,type IIIsecretion system, LPS, toxins and enzymes.

It causes melioidosis, skin ulceration and lymphadenoparhy

*B. pseudomallei* is an obligate aerobe, grows in various media, e.g. nutrient agar, blood agar and MacCon key agar. The colonies are typically rough and corrugated, similar to the colonies of *Pseudomonas stutzeri.* Ashdown's medium is used as a selective medium, where *it* produces wrinkled purple colonies

Important properties *that* differentiate it from *Pseudomonas stutzeri* include:

• Gelatin liquefaction positive

• Utilizes arginine

• Positive for intracellular polyβ hydroxy butyrate

***Burkholderla mallei***

*B. mallei* is a pathogen of horses. It causes glanders and farcy

*It* is similar to*that* of*B.pseudomallei*. However, *B.mallei* differs from*B.pseudomallei* in being:

- Non-motile
- Oxidase negative
- Inability to grow on MacConkey agar
- Does nor grow *at* $42^0$C

### 3.3.4.Genus *Stenotrophomonas*

*This genus contain many species, but only S.maltophila* has been known to cause human infections. It is an emerging nosocomial infection. There has been an increase in incidence of respiratory tract infections in patients of cystic fibrosis caused by *S.maltophila*[47]. The rate of isolation from these patients ranges about 10- 30%.

In a study done by Denton et al showed that clinical isolate of this bacterium mainly isolated from sputum samples – 26% [48]

**Diagnostic criteria**

- Oxidase negative

- Motile rod possessing polar multitrichous flagella.
- It decarboxylates lysine .
- Ferments 10% maltose
- Colonies may appear bluish on MAC agar and pale yellow or lavender on blood agar
- DNase, esculin and gelatin hydrolysis- positive.

*S. maltophilia* is usually susceptible to SXT, so this is the drug of choice for most infections.

### 3.3.5. Genus *Sphingobacterium*

*These are Gram negative rods containing large amount of Sphingophospholipids in the cell wall.* The important species in this Genus are *Sphingobacterium multivorum,S. spiritovorum, S. antarticum, S.faecium, S. thalophilum* and unnamed genomospecies 1 and 2. Among these, *S. multivorum* and *S. spiritovorum* are the two species are most frequently recovered from clinical samples.

They cause UTI, pneumonia, infection of the eye, wound , abscess ,and sepsis. These organisms are only rarely associated with serious infections[49] .

**Diagnostic criteria**

- Oxidase positive
- Non- motile
- Yellow pigmented colonies on Mac Conkey agar
- Indole negative
- Produce acid from glucose

- *Sphingobacterium* species are generally resistant to aminoglycosides and
- polymixin B.

They are susceptible to quinolones and trimethoprimsulfamethoxazole

### 3.3.6. Genus *Sphingomonas*

This genus consists of 23 species of which *Sphingomonas paucimobilis* and *S. parapaucimobilis* are thought to be clinically important [50].

It causes community acquired pneumonia, bacteremia and peritonitis are some of the infections reported to be caused by these organisms[51].

**Diagnostic criteria**

- Yellow-pigmented,
- Oxidase positive.
- Motile
- Esculin hydrolysis - positive

Most of the strains are susceptible to tetracycline, chloramphenicol, trimethoprim-sulfamethoxazole and aminoglycosides[52]

## 3.4. Carbapenems

Carbapenems are β lactams that contain fused β lactam ring system. It is a 4:5 fused lactam ring with a double bond between C2 and C3.[53] It is unsaturated and contains a carbon atom instead of sulfur atom at C1. The hydroxyethyl sidechain in carbapenems is important for its activity.

### 3.4.1. History

By 1976, the first β-lactamase inhibitors, olivanic acids which were natural products produced by the Gram-positive bacterium *Streptomyces clavuligerus was discovered*. Then in 1976,thienamycin was discovered from *Streptomycescattleya*.[54,55] These compounds were chemically unstable, so they were not used clinically.[Kattan *et al.*2008, Wallace *et al*. 2011].

Then a more stable thienamycin derivative, known as imipenem, was synthesized and approved for use in 1984 [Hellinger,1999]. However, imipenem is deactivated by dehydropeptidase -1 (DHP-1), found in the human renal brush border. Therefore,should be coadministrated with an inhibitor, cilastatin or betamipron in the ratio of 1:1 with imipenem to prevent hydrolysis by DHP-1 and reduced nephrotoxicity. [Kattan *etal*.2008, Wallace *et al*. 2011, Birnbaum J *et al*.1985]

Subsequently, carbapenems with better stability and broader spectrum such as meropenem, biapenem, doripenem and ertapenem were developed. [Bonfiglio *et al*. 2002]

### 3.4.2 Mechanism of action:

Carbapenems are not easily diffusible through bacterial cell wall. They enter the Gram negative bacteria through porin, then transverse periplasmic space and permanently acetylate the penicillin binding protein.[56]

### Classification

Carbapenems can be grouped into

**Group 1 :**

Includes broad spectrum carbapenems with limited activity against non fermenter Gram negative bacilli. It is suitable for community acquired infections. E.g. ertapenem

**Group 2 :**

Includes broad spectrum carbapenems with activity against non fermenter Gram negative bacilli. It is suitable for nosocomial infections. E.g. imipenem, meropenem and doripenem,

**Group 3 :**

Carbapenems with activity against methicillin resistanat Staphylococcus aureus

### 3.4.3.Microbiological spectrum of activity

Carbapenems have a broad spectrum of activity. They are the drug of choice for the treatment of infections caused by ESBL producing Enterobacteriaceae. [Rodrigues,2011]. Carbapenems except ertapenem are active against clinically significant Gram-negative non-fermenters such as *P. aeruginosa*, *Burkholderia cepacia* and *Acinetobacter* spp. [Unal *et al.*2005]. They are also active against streptococci, methicillin-sensitivestaphylococci, *Neisseria* and *Haemophilus*[57].

### 3.4.4. Mechanisms of carbapenem resistance

Mechanisms of resistance to carbapenems include

- production of β-lactamases
- efflux pumps
- mutations that alter the expression and/or function of porins and PBPs.

Combination of these mechanism causes high level resistance.

In Gram-positive cocci , resistance is due to the result of substitutions in amino acid sequences of PBPs

**Carbapenemases hydrolyzing enzymes:**

Carbapenamases are Carbapenem hydrolyzing enzymes. They are present in the periplasmic space. They are broadly divided into two types based on the reactive site of the enzymes;

- Serine carbapenemases
- Metallo-β-lactamases.

Presently, β-lactamases are classified into four distinct classes based on structural similarities (classes A, B, C,and D) or four groups based on hydrolytic and inhibitor profiles. Class B β-lactamasesuse zinc to inactivate β-lactams. Class A, C, and D β-lactamases use a serine as a nucleophile to hydrolyze the β-lactam bond.

KPC and GES enzymes belong to class A carbapenemases,, VIM, IMP, and NDM types belong to class B carbapenemases and OXA belongs to class D carbapenemases. These enzymes are encoded by mobile DNAelements with high capacity for dissemination. [Walsh 2008,2010].

### 3.5. Metallobetalactamases

MBLs contain a set of amino acids with two zinc ions at their active site. The zinc ions have two water molecules which is essential for hydrolysis. The major zinc-binding site is histidine-X- histidine-X-aspartic acid (HXHXD). These two zinc binding sites function separately with the primary zinc binding site assisted by the secondary site. Exception to this is B2 enzymes because this class B2 enzymes have only one zinc ion at their active site.

These MBL enzymes contain a αββα site with a central β and two helices on each side. The zinc ions are held in place by three histidines molecule and a water molecule. The water molecule plays a critical role in catalysis of the antibiotics.

The zinc in the active site positions the β-lactam bond. This enables nucleophilic attack by zinc-bound water and hydrolysis. These enzymes are resistant to the β lactam inhibitors such as clavulanic acid and sulbactum. They will also not hydrolyze monobactam such as aztreonam.

The cardinal features of metallo β lactamase are

- Class B metallo β lactamases use a $Zn^{2+}$ cation for hydrolysis of the β-lactam ring.
- They hydrolyse all β-lactam antibiotics like penicillins, cephalosporins and even carbapenems except monobactam.
- They are susceptible to ion chelators such as ethylene diamene tetraacetic acid (EDTA) and thiol compounds

- They are not inhibited by the β-lactamase inhibitor such as clavulanic acid.

### 3.5.1. CLASSIFICATION OF METALLO-β-LACTAMASES:

**Depending on the zinc requirement**:

Depending on the requirements for zinc, metallo β lactamases can be classified into three different subclasses

- B1 Enzymes
- B2 Enzymes
- B3 Enzymes

- **B1 enzymes**: Fully active with one or two zinc ions. These include IMP-1, VIM-2, and CcrA.
- **B2 enzymes**: These enzymes are inhibited by the addition of second zinc ion. The example is CphA.
- **B3 enzymes**: These enzymes require two zinc ions. Eg: L1

**Depending on the carbapenem hydrolyzing capacity:**

On the basis of carbapenem hydrolyzing property, class B enzymes are divided into three groups

**3a:**

- It has a broad spectrum activity against carbapenem.
- It has property of binding of two zinc atoms for optimal hydrolysis.
- Three molecules of histidine are present in the one binding site of the zinc and asparagine, cysteine and histidine are present in the second binding site of zinc in the enzyme.

- The examples include BCII, IMP-I, Ccr A, VIM, GIM, SPM-1.

**3b:**

- It has preferential activity towards carbapenem.
- The addition of second zinc atom is inhibitory to the catalytic activity of the enzyme
- It has two molecules of histidine and one molecule of asparagine in the first binding site of the zinc atom.
- The examples include Cph A, Sfh-1.

**3c:**

- It has high specificity for hydrolyzing cephalosporins but has poor activity against carbapenem.
- It has three histidine molecules in the first binding site and two histidine and a asparagine molecule in the second binding site which will be helpful in the optimum hydrolysis of the antibiotics.

**Depending on the location of the MBL gene:**

According to the location of MBL gene they are classified into two types,

- Transferable MBLs
- Chromosomally mediated MBLs

**Chromosomally Mediated MBLs:**

Most of the MBL are chromosomally encoded. Their expression can be constitutive or inducible. In *B. cereus, Stenotrophomonas maltophilia*, *Aeromonas hydrophila,* and *Aeromonas jandaei* the metallo β lactamases are inducible.

These bacteria show high level resistance to β lactams. As these MBL genes are chromosomally encoded, they are not easily transferred.

**TRANSFERABLE MBLs**:

The Transferable MBLs are located in the integrons.

Integron is a mobile genetic elementthat possess a site, attI, at which additional DNA in the form of gene cassettes, can be integrated by the site specific recombination and which encodes an enzyme ,integrase that mediate these site specific recombination events.

These integrons which carry multiple resistance gene cassettes can be readily mobilized. Hence they are significant in the spread of antibiotic resistance.

Integrons capture antibiotic resistance gene cassettes by using a site-specific recombination system. There are 5 distinct integron classes that are associated with antibiotic resistance gene cassettes. Among the five, three major classes of integrons class1, class2 and class 3 have been described for gram-negative bacteria.

Integrons which have β -lactamases are commonly present in *A. baumannii, P. aeruginosa,* and other species of gram negative bacteria included in Ambler class A, B, and D β –lactamases.

Integrons are found to be an important source for the spread of *bla* gene. The *bla* gene types like IMP-1 to IMP-4, IMP-6 to IMP-8, IMP-12, VIM-1, VIM-2, and GIM-1 are also included in integron encoded β lactamases.

The Transferable MBLs are [58],

i. IMP ( “Imipenemases”)
ii. VIM (“Verona integron-encoded metallo β lactamase”)
iii. SPM (“Sao Paulo metallo β lactamase”)
iv. GIM (“German imipenemase”)
v. SIM (“Seoul imipenemase”).

**IMP ( “Imipenemases”)**

These are the first acquired MBLs identified and were detected in *Pseudomonas* spp., *Acinetobater* spp., and *Enterobacteriaceae* [Zhao*et al*. 2011b].. Since then, 33 IMP variants have been assigned and IMP type carbapenemase producers have spread worldwide. The endemic spread of IMP type enzymes has been reported from Japan, Taiwan, East of China, and more recently from Greece, although outbreaks and single reports have

been reported in many other countries. It has been suggested that selection of IMP type genes occurred first in Japan as a consequence of heavy carbapenem usage. These resistance genes encode decreased susceptibility to unrelated antibiotic molecules such as otherbetalactams, aminoglycosides, sulfonamides, and chloramphenicol. They are usuallyidentified inside transposon structures, thus allowing their spread. [Zhao *et al.* 2011b,Nordmann *et al*.2012b, 2011a]

**VIM ("Verona integron-encoded metallo β lactamase")**

Another family of MBLs includes the VIM enzymes. VIM-1 was first identified in Italy in 1997, and shortly after the VIM-2 variant was reported in Francefrom a *P. aeruginosa* isolate [Walsh *et al*.2005]. The VIM group of enzymes contains33 variants, mainly identified from *P. aeruginosa* and Enterobacteriaceae isolates. The genes also correspond to gene cassettes located inside class 1integrons.

VIM-2 is the most common MBL type reported worldwide, with an endemic spread in Southern Europe and Southeast Asia. [Zhao *et al*.2011a]

**SPM(Sao Paulo MBL):**

SPM-1 was first isolated in a *P. aeruginosa* strain in Sao Paolo, Brazil. Since the initial report, single clones of SPM-1-containing *P. aeruginosa* have caused 33 multiple hospital outbreaks with high mortality in Brazil. [Marra *et al.* 2006, Poirel *et al*. 2004, Zavascki *et al*.2005, Toleman *et al*.2002]

**GIM(German imipenemase):**

GIM-1 was isolated in Germany in 2002. GIM had approximately 30% homology to VIM, 43% homology to IMPs, and 29% homology to SPM. GIM-1 has characteristics similar to those of the other acquired MBL in that it was found in five clonal *P. aeruginosa* isolates within a class 1 integron on a plasmid.[ Castanheira *et al*. 2004a, Strateva *et al* 2009]

**NDM-1 (New Delhi Metallo βlactamase):**

At the Military Medical Academy in Serbia, routine analysis of carbapenemase producing bacterial isolates, revealed NDM-1 in seven clinical isolates of *P.aeruginosa.* The source patients were hospitalized in Serbia and had no history of travel to any other country. Subsequently, in 2012 France reported recurrent pyelonephritis due to NDM-1 producing *P. aeruginosa.* This patient had history of prior hospitalization in Serbia and gave rise to the hypothesis that the Balkan statesmay be endemic for NDM-1 producers [Jovcic *et al* 2011, Nordmann *et al* 2011b, Flateau *et al* 2012].

## 3.6. PHENOTYPIC METHODS FOR MBL DETECTION:

A resistant phenotype or decreased susceptibility to carbapenems indicates the possibility ofcarbapenemase production. CLSI suggested that it better not to detect carbapenemases routinely but instead to reduce carbapenem breakpoints so that a susceptible test predicts effective therapy. However, detection tests may be done for epidemiological and academic purposes.

**Screening**

Only meropenem and imipenem are recommended for testing against *P. aeruginosa* and *Acinetobacter spp*.

The susceptibility breakpoints recommended by CLSI (2017; M100-S21)

- For isolates of *Acinetobacter* sp., an imipenem MIC of ≥ 8µg
- For isolates of *P. aeruginosa,* an imipenem MIC of ≥16 µg.

The zone diameter by disc diffusion method defined resistant pattern when the zone diameter is

- ≤ 15mm for imipenem(10 µg) and meropenem(10 µg) in case of P.aeruginosa
- ≤ 13mm for imipenem(10 µg) and meropenem(10 µg) in case of Acinetobacter

Since imipenem disc test performs poorly as a screen for carbapenemases, CLSI recommends usage of meropenem discs.

**Phenotypic detection of carbapenemase activity**

Metallo beta lactamases produced by nonfermenters posses a serious problem due to their ability of transmission . There are no CLSI guidelines in the detection of MBL , even then there are many different methods to detect MBLs which may be performed in the laboratory.

### 3.6.1. Modified Hodge test:

MHT or the clover leaf method has been extensively used as a general phenotypic method for the detection of carbapenemase activity. This test does not differentiate between serine and metallo-beta-lactamases. [Pasteran *et al.*2009].

Make 0.5 McFarland of standard suspension of *E. coli* ATCC 25922 (indicator organism) in normal saline.

Make 1:10 dilution of the above suspension.

With the help of sterile swab stick, make a lawn culture on MHA plate.

A 10µg meropenem/10 µg ertapenem disk is placedin the middle of the agar plate.

Interpretations of the diameters of zone of inhibitionare as follows

**Modified Hodge test positive test**

It has a clover leaf‐ like indentation of the *E. coli* 25922growing along the test organism growth streak within the disk diffusion zone indicating that this isolate is producing a carbapenemase .

**Modified Hodge test negative test**

It has no growth of the *E. coli* 25922 along the test organism growth streak within the disc diffusion indicating that this isolate is not producing a carbapenemase.

**Quality Control**

• Positive control: *K. pneumoniae* ATCC BAA 1705

• Negative control: *K. pneumoniae* ATCC BAA 1706.

**3.6.2. Double disc synergy test**

It is also called as disc approximation test. It is often used to detect MBL producers. This test relies on inhibition of MBL by EDTA10 µl of 0.5 M (750µg ). The test isolate is inoculated on Mueller Hinton agar plate to produce a semiconfluent growth. Two discs are placed at a distance of 20-30 mm; one containing beta-lactam and the other containing EDTA. After incubation, enhancement of the zone of inhibition in the area between Imipenem and the EDTA disc in comparison with the zone of inhibition on the far side of the drug is interpreted as a positive result.

Imipenem, ceftazidime andcefepime have been used as the betalactam drug for this test. Ceftazidime mercaptopropionicacid, ceftazidime-clavulanate-EDTA and cefepime-clavulanate-EDTA too have been tried to detect MBLs.

Kalantar E, et al, detected 22 MBL positive isolates out of 100 *P. aeruginosa* (22%) by DDST method [59].

In a study conducted by Bogiel T, et al about 72.9% of the isolates are positive for MBL by DDST from 67 carbapenam resistant strains isolated from intensive care units[60].

### 3.6.3. Combination disc methods

This method is more accurate in identifying the carbapenemase type. It involves the use of two discs; one with a carbapenem antibiotic and the other with carbapenem with an inhibitor. An increase in the zone diameter around the disc with inhibitor by 7mm or above over the carbapenem disc indicates positive carbapenemase production.

Valenza G, et al in 2010, suggested that the CDT is a valid alternative to the molecular investigation of MBL detection from a study done at German university hospital[61].

A study was conducted by P Pandya et al 2011, on evaluation of phenotypic methods on MBL detection in gram negative bacilli. He found that out of 450 *P. aeruginosa* isolates about 27 were positive for MBL production. MBL production was highest in *Pseudomonas* (9.92%) than other gram negative bacilli. The CDT detected 26 isolates (96.30%) and DDST detected 22 isolates (81.48%) as MBL producer. He concluded that the CDT is the most sensitive method for detection of MBL production in gram negative bacilli. DDST has subjective variation in interpreting the results. The results were more obviously interpreted in CDT than DDST[62]

### 3.6.4.E test MBL strips

E test strips are available as imipenem and imipenem-EDTA combinations. The strip contains imipenem gradient (256-4 µg/ml) and imipenem+EDTA (64-1µg/ml +320µg/ml). A threefold-or-greater decrease in the imipenem MIC in the presence of EDTA indicates MBLproduction. False-negative results have been reported when an isolate is having an imipenem MIC of <4 µg/ml. False positive results can occur with some bacteria as EDTA alone has inhibitory action due to permeabilization of the outer membrane

In a study by Kaleem F et al reported that MBL E test can be used to confirm the MBL[63].

Van der Bij et al compared CDT with the E test for the MBL production. The author concluded the sensitivity and the specificity for the E test was 100% and 95% respectively [64].

Saha R et al in their study reported 48% MBL producers by E test from *P.aeruginosa*[65].

### 3.6.4. Genotypic methods:

**DNA hybridization:**

Colony blot hybridizations using labeled probes have been used to efficiently screen large numbers of clinical isolates for carbapenemase genes. Southern blot hybridization too hasbeen used to determine whether the carbapenemase gene resides on a plasmid or the chromosome.

**Polymerase chain reaction (PCR)**

It is used to rapidly identify the carbapenemase family using specific primer pairs. Mere amplification of the genes do not reveal the complete identity of thecarbapenem type, hence complete sequencing of the gene is undertaken.

**3.7. Treatment of infections caused by Carbapenem resistant NFGNB.**

Early recognition and treatment of carbapenem-resistant species must become a clinical priority for all hospitalized patients as resistance to carbapenems is often associated with resistance to other classes of antibiotics such as aminoglycosides and fluoroquinolones. Unusually elevated MICs to carbapenems should arouse suspicion for a carbapenem-resistant isolate and preclude the use of carbapenems even if the MICs do not exceed the breakpoints forresistance. Carbapenems as last-resort antibiotics recommended in eliminating ESBL-producing Enterobacteriaceae could no longer be a viable choice as monotherapy.

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**Antibiotic Options**

**Aztreonam:**

It is stable to metallo-carbapenemases, including IMP, VIM and NDM.However, for isolates that also co-produce AmpC or ESBL, aztreonam is ineffective.[Karthika *et al*.2009, Walsh *et al*.2005]

**Sulbactam:**

Sulbactam is active against *A.baumannii* by inhibiting PBP-2.In most countries it is available as a co-formulation with ampicillin.Sulbactam is useful in the treatment of

carbapenem resistant *A.baumannii* infections in combination with colistin.[Kosmidis *et al*.2012].

**Tigecycline:**

It is a tetracycline analogue and is the first glycylcycline to be launched for clinical use. It acts by inhibiting the protein synthesis in the bacterial cell by binding to the 30S subunit of the ribosome. Its low serum concentrations compromiseits use in bloodstream infections. It is not useful in treatment of nosocomial pneumonia as indicated by poor results in the study of ventilator associated pneumonia. Despite tigecycline being one of the first-line agents for use in the setting of carbapenemase-producing isolates, clinical failures have been reported in the literature as it is affected by the intrinsic multidrug pumps of *P. aeruginosa* and Proteae and therefore it is not useful to treat infections caused by them.[Kanj *et al*.2011]

**Polymyxin:**

Given limited therapeutic options, clinicians have returned to the use ofpolymyxin B or polymyxin E (colistin) for the most carbapenem resistant gram negative infections. Polymyxin B differs from colistin by only one aminoacid. These drugs acts by disturbing the bacterial cell membrane, thus increasing permeability, leading to cell death. Although colistin retained activity against carbapenemase producing NFGNB in initial studies, more recent data suggest that resistance to colistin is emerging, and outbreaks of colistin-resistant strains have been reported..[Kanj *et al*.2011,Peleg *et al.* 2008, Gupta *et al*.2011]

**Fosfomycin:**

Fosfomycin inhibits bacterial cell wall synthesis, thereby exhibiting bactericidal activity against gram-positive and gram-negative pathogens. It is useful for the treatment of uncomplicated urinary tract infections at a single oraldose. The emergence of resistance among GNB has sparked new interest in using fosfomycin to treat infections caused by MDR isolates.[Kanj *et al*.2011]

**Rifampin:**

In vitro studies suggest that rifampin has a synergistic activity when used as part of a combination therapy regimen against carbapenemase-producing *E coli, Kpneumoniae* and *A. baumannii.*[Kanj *et al*.2011]

**Extended-Infusion Strategy for β-Lactams**:

Carbapenems have also been evaluated in extended-infusion regimens. Lengthening meropenem infusions from 30 minutes to3 hours was found to be advantageous with isolates of *P. aeruginosa* and *Acinetobacter* species with intermediate resistance. This benefit was not observedwith resistant isolates having very high MICs.[Peleg *et al.* 2008, Kanj *et al*. 2011,Manchanda *et al*. 2010, Chambers *et al*. 2005]

**3.8.1.Global scenario of carbapenem resistance in *P.aeruginosa***

According to SENTRY study, in Brazilian hospitals ,P.aeruginosa was themost frequently found bacterium in lower airway infections displaying a resistance of 49% to imipenem. During European MYSTIC study, 31.8% of *P. aeruginosa* strains resistant to carbapenems, 7.0%in USA, 54.3 in Turkey, 6.7% in UK. Russian study showed that 22.9% of *P.aeruginosa* strains were resistant to imipenem.

The prevalence ofimipenem- resistant *P. aeruginosa* in Tehran has been reported to be within the rangeof 16% to 100%. Shahid *et al*. found 100% MDR in *P. aeurginosa* burn isolates inIndia in 2003.[Shahid *et al* 2012].

Various studies were conducted in India to detect the prevalence of MBL producers. In 2010, at Bijapur, Prashant Durwas Peshattiwar et al reported that about 7.8% of *P.aeruginosa* was MBL producers[66]. According to Deeba Bashir et al from Kashmir ,the prevalence of MBL producing *P.aeruginosa* were about 11.66%.[67]In a study, Agrawal et al from Nagpur concluded that about 8.05 % of the *P.aeruginosa* produces the metallo beta lactamase enzyme. Seema Bose et al from Maharashtra in their study reported that 15.71% were MBL producers[63].

### 3.8.2.Global scenario of carbapenem resistance in *A. baumannii*

Surveillance studies showed that the percentage of carbapenem-resistant *Acinetobacter* species gradually increased over the last ten years in Europe, North America, Asia and Latin America. Developing countries such as Morocco, Thailand, India, and Indonesia also had outbreaks caused by carbapenem-resistant *A. baumannii* .

According to a report from a teaching hospital in Spain (2002), the prevalence of imipenem-resistant *Acinetobacter* spp. had increased from no resistance in 1991 to 50% in 2001. According to[Greek System for Surveillance of Antimicrobial Resistance (GSSAR), , the proportion ofimipenem-resistant *A. baumannii* isolates from patients hospitalized between 1996and 2007, in tertiary care hospitals, in several regions of the country rose from no resistance to 85% (ICUs), 60% (medical wards), and 59% (surgical wards) in Greece. The prevalence of imipenem resistance in *Acinetobacter baumannii* isolated from

a burns unit of USA(2007) was found to be as high as 87% [Manchandra *et al.* 2010, Zarrilli *et a.l* 2009,Gladstone*et al* 2005.]

In India Gladstone *et al*. (2005)f rom Vellore, India (2005), reported a prevalence of 14% carbapenem resistant *Acinetobacter* spp., isolated from tracheal aspirates . In 2006, at Delhi, the prevalence of carbapenem resistance in *Acinetobacter* spp. isolated fromdifferent clinical samples was found to be almost 35%.

# 4.MATERIALS AND METHODS

- The present study was undertaken at the Department of Microbiology , Tirunelveli Medical College for a period of one year from June 2016 – July 2017.
- This was a prospective cross sectional study.
- This study was aimed to isolate and identify 100non fermenter Gram negative bacilli from clinical samples and to detect *bla* VIM mediated carbapenamase resistance among the isolates

## 4.1. Materials

A total of 100 non fermenter Gram negative bacilli were collected from various clinical samples such as pus, urine, burn, wound, sputum, pleural fluid and CSF were taken in the study. All samples were collected under aseptic precautions by standard procedures and processed according to standard guidelines

### 4.1.1 Inclusion criteria

1. Specimens from patients admitted in wards were only included.
2. All isolates of non fermentor Gram negative bacilli confirmed by biochemical reactions
3. Isolates showing resistance to Meropenem were only tested for production of carbapenamase

### 4.1.2. Exclusion criteria

Specimens from outpatient department

### 4.1.3.Ethical clearance

The study was started after getting ethical committee clearance from the institution

### 4.1.4Informed consent

Informed consent was obtained from all patients included in the study.

### 4.1.5. Proforma:

The proforma was filled with the details like name, age, sex, ward, clinical diagnosis, risk factors, undergone any surgery, duration of hospital stay and other parameters significant to the present study.

### 4.1.6. Sample storage

The isolated non fermenter Gram negative bacilli were sub-cultured on to nutrient agar slope and stored at $+2^0$ to 8˚C. The isolates were sub-cultured every fortnight

### 4.1.6. Safety precautions

All the procedures were carried out in biological safety cabinet (type 2) with universal precautions.

## 4.2.METHODS

### Collection and Processing Of Various Samples

All the samples were collected under aseptic precautions by standard procedures. They were then processed according to the standard guidelines. The specimens were inoculated on to nutrient agar(NA) MacConkey agar (MA) and Blood agar(BA). The media were incubated at $37^0$C overnight. Plates were examined for growth.

### 4.2.1. Culture identification

Organismsthat failed to acidify the butts of triple sugar iron media were considered nonfermenters and were subjected to a battery of tests

The most commonly isolated nonfermenters i.e., *P. aeruginosa, A. baumannii* and *S. maltophila* were identified by the following characteristics:

***Pseudomonas aeruginosa* was identified by the presence of :**

- Slender Gram negative rod
- Motile
- Catalase positive
- Oxidase positive
- β-hemolytis on sheep blood agar (SBA) and will produce flat spreading colonies with a characteristic metallic sheen.
- Fruity,grapelike odor caused by the presence of 2-aminoacetophenone.
- Denitrification of nitrates and nitrites,
- Arginine dihydrolase (ADH)–positive,
- Growth at 42° C – positive
- Citrate utilization –positive

***Acinetobacter baumannii* was identified by following characteristics**:

- Oxidase negative
- Catalase positive

- Nonmotile.
- Colonies on blood agar appear small to medium sized, smooth, translucent to opaque with entire edges.
- On MacConkey agar, produce pale orslightly pinkish colonies.
- Coccoid or coccobacillary appearance on Gram stain
- Penicillin resistance

***Stenotrophomonas maltophila* was identified by:**

1. Oxidase test negative
2. Production of acid in OF maltose
3. Lysine decarboxylase positive
4. Motile

### 4.2.2. ANTIBIOTIC SUSCEPTIBILITY TESTING:

All the isolates were subjected to antibiotic susceptibility testing by Kirby Bauer disc diffusion method according to CLSI guidelines.[68]

Kirby-Bauer's disc diffusion method:

About 3 -5 colonies of the test organism was picked up with sterile loop and suspended in peptone water and incubated at 37°C for 2 hours. The turbidity of the suspension was adjusted to 0.5 McFarland's standard (1.5 X $10^8$ CFU/mL) using Wickerham's chart. It was then spread on the surface of a cation-adjusted Mueller-Hinton agar (MHA) plate using sterile cotton swab. The panel of antibiotic discs was applied and incubated at 37˚C for 18-24 hours. The zone size was recorded and interpreted as per the CLSI guidelines 2016.[69]

All the antibiotic disc used were procured from Hi-media , Mumbai. Discs were stored in a tightly sealed container in refrigerator at $+2^0$- $8^0$ C. Before using the disc, disc were allowed to equilibrate at room temperature.

The diameter of the zone of inhibition was measured and interpreted according to the CLSI 2016 guidelines.

**4.2.3. Methods to detect carbapenem resistance**

**4.2.3.1. Screening by disc diffusion method with meropenem**

All the isolates of NFGNBwere tested for the susceptibility for Meropenam by disc diffusion method as per the CLSI guidelines.

About 3-5 colonies from the 24 hour young culture is inoculated in the peptone water and incubated for 2-4 hours and then adjusted to 0.5 Mc Farland standards suspension.

Using a sterile cotton swab the inoculum was inoculated on to the Muller Hinton agar by streaking evenly over the surface of the agar in three directions with the plate rotated approximately 60° for even distribution.

The plate was dried for 3-10 minutes before applying the Meropenem disc using sterile needle.

The disc was placed 15mm from the edge of the plate.

The plate was incubated at a temperature of 37˚C for 16-18 hours.

According to the CLSI guidelines the zone of inhibition was measured and interpreted. For Meropenem disc, zone size of ≤ 15mm was taken as resistant for

pseudomonas and other species and zone size of ≤ 13mm was taken as resistant for Acinetobacter.

**4.2.3.2. Phenotypic methods to detect carbapenemases**

The isolates which were found to be resistant to Meropenem were selected and subjected to various phenotypic methods like CDT, DDST, MBL E Test to detect MBL in test organism and then are confirmed genotypically by PCR.

**4.2.3.2.1.Combined Disc Test :**

**Preparation of EDTA solution:**

Dissolve 18.61g of disodium EDTA.2H2O in 100 ml of distilled water.

By using sodium hydroxide (NaOH) the pH was adjusted to 8.0.

The solution was autoclaved.

This is the solution of 0.5 M ethylene diaminetetra acetic acid containing 750 µg of EDTA.

For the procedure, 10 µl of 0.5 M EDTA solution was used.

**Combined Disc Test procedure:**

The 24 hr young culture isolate of the test strain was inoculated on to the Muller Hinton agar plate.

Two Meropenem discs 10 µg were placed on the dried agar plate at a distance of 50 mm.

To one of the disc 10µl (750 µg) of 0.5 M EDTA solution was added.

The plate was incubated at 37˚C aerobically for 16-18 hours.

The zone of inhibition was measured for the Meropenem and the Meropenem EDTA combined disc.

The test is considered positive for the detection of MBL enzyme production when there is increase in the diameter of the zone by more than or equal to 7mm (≥7mm) for the Meropenem EDTA combined disc

**4.2.3.2.2.Meropenem-EDTA Double Disc Synergy Test (DDST):**

The test strain was inoculated as lawn culture on to the Muller Hinton agar plate as per the CLSI guidelines.

The Meropenem (10 µg) disc was placed 10 mm apart from the sterile blank disc.

The sterile blank disc was added with 10 µl of EDTA

The plate was incubated at 37˚C for 16-18 hours.

The enhancement of the zone of inhibition towards EDTA disc was considered positive for MBL production of the test strain.

**4.2.3.2.3. MBL E Test:**

Make 0.5 McFarland suspension of the test strain in normal saline

• With the help of sterile swab , test strain is inoculated as a lawn on the MHA plates

• Place the E test strips (mixture of Meropenem + EDTA and Meropenem in a concentration gradient manner) on the plate.

The strip was then taken with a sterile forceps or E test applicator and applied to the dried agar surface with the MIC scale facing upwards.

• Incubate the plate at 37°C for 24 h

• The MIC for MP/MPI ≥ 8 or deformation of ellipse or phantom zone was considered positive for MBL production.

#### 4.2.3.3. Genotypic method

**PCR**

The Meropenem resistant NFGNBisolates were further tested for *bla*VIM gene by Real-Time PCR. The PCR kit was procured from Helini Biomolecules, Chennai, India. According to the manufacturer's instructions, the procedure was performed.

**DNA Extraction:**

This procedure yields purified DNA of more than 30kb in size obtained after the lysis of the cell. DNA was extracted using the silica based membrane technology in the form of a spin

**Storage and stability:**

The bacterial genomic DNA extraction kit was stored at room temperature.

The proteinase K and Lysozyme were stored at -20 $^{0}$ C.

**Principle**

The cells were lysed with the enzyme Proteinase K and the nucleases were inactivated by chaotropic salt. The nucleic acids were bound to special silica fibres in the spin column tube. The cellular components contaminating the bound nucleic acid was removed in a series of rapid "wash and spin" steps. The elution releases the nucleic acids from the silica fibre.

**Bacterial pellet preparation:**

About 4-6 colonies of the test strains were inoculated in peptone water and incubated at 37$^{0}$C overnight.

1-1.5 ml of bacterial culture was transferred into the sterile 2ml centrifuge tube. The tube was centrifuged at 8000rpm for 5 minutes at room temperature.

The supernatant was discarded and the pellet was used for the DNA extraction.

**DNA extraction procedure:**

1. The bacterial pellet was suspended in 200 µl phosphate buffer saline and the suspension is vortexed briefly.
2. 180 µl of digestion buffer and 20 µl of lysozyme were added and vortexed for 10 seconds.
3. The mixture is incubated for 15 minutes at $37^0$C.
4. 200 µl of binding buffer, 20 µl of proteinase K and 5 µl of internal control template was added and mixed well by pulse vortex. It was incubated in a water bath at $56^0$C for 15 min.
5. 300 µl of Isopropanol is added and mixed well.
6. The entire sample was pipetted into the spin column. At 12,000rpm it was centrifuged for 1 minute. The flow through was discarded. The spin column was placed into the collection tube.
7. 500 µl of 7% ethanol is added to the spin column. At 12,000rpm it was centrifuged for 1 minute. The flow through was discarded. The spin column was placed into the collection tube. This step was done twice.
8. The empty spin column with the collection tube was centrifuged at 12,000rpm for 2 minutes to avoid residual ethanol.

9. The collection tube is discarded and the spin column was placed in to a fresh micro centrifuge tube.

10. 75 µl of the pre-warmed (warmed at $56^0$C) Elution buffer was added to the centre of the spin column and incubated for 2 minutes at room temperature. It was centrifuged for 1 minute at 13,000rpm.

The spin column was discarded and eluted DNA was taken for the PCR procedure

**PCR Amplification:**

*bla*VIM primer & probe mix:

The *bla*VIM primer & probe mix consists of TaqMan probe which is florescent labeled with FAM (Fluorescein amidite), forward primer and reverse primer.

For VIM gene:

**Forward primer:**

CGCGGAGATTGAGAAGCAAA

**Reverse primer:**

AGCCGCCCGAAGGACATC

**Probe sequence:**

TTGGACTTCCTGTAACGCGTGCA

**Internal Control primer & probe Mix**

The internal control primer & probe mix consists of TaqMan probe which is florescent labeled with HEX( Hexachloro Fluorescein), forward primer and reverse primer. The internal control is included to make sure that PCR inhibitors removed during the DNA

extraction procedure and the performance of PCR mix ingredients are good. The internal control if amplified shows that PCR inhibitors are not present in the sample and the nucleic acid purification is optimum. It helps to rule out false negative results.

**Positive control:**

*bla*VIM positive template is used as positive control. A positive result indicates that the primers and probes worked correctly in that particular experiment.

**Negative control:**

Nuclease free water as used as a negative control. A negative result indicates that there is no contamination in the reagents.

**Materials required:**

- Real time PCR equipment with FAM/JOE channel
- 0.2ml PCR tubes/8well strips
- Micropipette and tips

**PCR detection mix:**

The *bla*IMP and *bla*VIM reaction mix for the samples consisted of probe PCR master mix 10µl, *bla* IMP primer probe mix 10µl, *bla*VIM and internal control primer probe mix 10µl, purified DNA sample 5µl and a total volume of 25µl.

For positive control mix, 5µl of positive control template was added instead of sample DNA and for negative control mix, 5µl of nuclease free water was added instead of sample DNA.

The negative control is added first followed by samples and finally positive control was added to prevent cross contamination.

The PCR detection mix was centrifuged after adding all the ingredients and they were placed in the thermo cycler where the PCR reaction was allowed to occur .

**Table 1. *bla* VIM reaction mix for samples**

| S.NO | Components | Volume |
|---|---|---|
| 1 | Probe PCR master mix | 8 µl |
| 2 | Taq enzyme mix | 2 µl |
| 3 | Bla VIM Primer Probe mix | 2.5 µl |
| 4 | Internal control Primer probe mix | 2,5 µl |
| 5 | Purified DNA sample | 10 µl |
| 6 | Total reaction volume | 25 µl |

**PCR amplification steps:**

**Initial Denaturation:**

This is the first step in the amplification procedure. The thermocycler raises the temperature to 95˚C for five minutes for Taq enzyme activation.

**Denaturation:**

The temperature is elevated to 95˚C for 20 seconds. The double stranded template DNA gets separated into two complementary strands.

**Annealing:**

When the temperature is decreased to 55˚C for 20 seconds the complementary binding of the two specific oligonucleotide primers to the DNA template take place.

**Extension:**

The DNA polymerase extends the primers when the temperature is increased to 72˚C for 20 seconds. The template DNA is synthesised using deoxynucleotides (dNTPs) in the reaction mixture. Two single stranded DNA templates and newly synthesized complementary DNA strands attach together to form new double stranded DNA copies. Every copy of newly formed DNA may function as a template for further amplification. The products will be amplified in an exponential manner in each cycle. At the end of 40 cycles, the final PCR products will have 2n copies of template DNA. Data analysis was made at the end of extension and the computer produces the cross threshold (Ct) value by calculating the fluorescence emitted at the end of each cycle.

**Table:2. Amplification profile for *bla*VIM gene:**

| | Step | Time | Temperature |
|---|---|---|---|
| | Taq enzyme activation | 15 min | $95^0$C |
| 45 cycles | Denaturation | 20 sec | $95^0$C |
| | Annealing | 20 sec | $56^0$C |
| | Extension | 20 sec | $72^0$C |

VIM = FAM channel

Internal control = HEX channel

Interpretation of the results:

**Negative control:**

The negative control reactions should not exhibit fluorescence growth curves that cross the threshold line.

**Positive control:**

Positive control reactions should produce fluorescence growth curve before 40 cycles.

**Test sample positive:**

When all the controls fulfill the quantified requirements, a test sample is measured positive. Any samples which exhibit fluorescence that cross the threshold line at or after 38 cycles should be retested

**Test sample negative:**

When all controls fulfill the quantified requirements, a test sample is measured negative.

Internal control interpretation:

According to the protocols (assuming 100% extraction efficiency) the CT value is expected within 24-31 for the internal control. This may differ depending on the extraction efficiency, the quantity of elute added to the PCR reaction and the settings of machine. If the amplifying sample had high genome copy number then the internal control may not produce an amplification plot. This can be interpreted as positive result rather invalidates it.

**Table 3.Interpretation of results**

| **Test sample** | **Negative control** | **Internal control** | **Positive control** | **Interpretation** |
|---|---|---|---|---|
| Positive | Negative | Positive | Positive | Positive |
| Negative | Negative | Positive | Positive | Negative |
| Negative | Negative | Negative | Negative | Repeat |
| Positive | Positive | Positive | Positive | Repeat |

# 5.RESULTS

## 5.1. Study description

The study entitled “Isolation and identification of Non fermentor GNB and detection of *bla* VIM mediated carbapenamase resistance from clinical isolates in a Teriatary care Hospital ” was carried out in the department of Microbiology, Tirunelveli Medical College. A total of 100 isolates of Non Fermenter Gram Negative Bacilli were isolated from specimens of respiratory tract infection, wound infection, urinary tract infections, septicaemia, post operative wound infections, ear infections satisfying both inclusion and exclusion criteria. The isolated NFGNB were subjected to meropenem resistance by disc diffusion test and carbapenemase production was tested by both phenotypic and genotypic methods. The susceptibility pattern to other antibiotics were also analysed.

The demographic profile of the study population were also analysed.

## 5.2. Statistical Analysis:

Data regarding the subjects were defined in terms of percentages. The statistical measures were completed with the help of the statistical software IBM SPSS statistics 20. The p values < 0.05 was considered as significant ($p < 0.05$) in Chi square and Mc Nemer test.

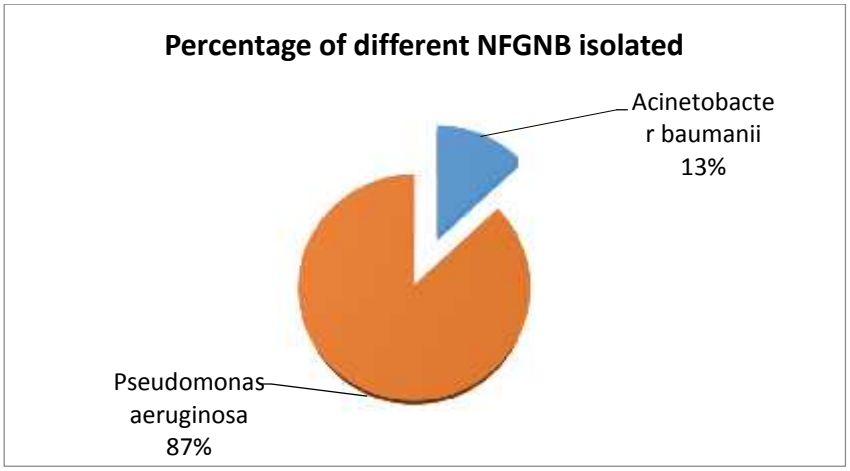
## 5.3.Percentage of different types of NFGNB

From the 100 NFGNB, the different types of NFGNB isolated were as follows. Pseudomonas aeruginosa was the most common isolate (87%) followed by Acinetobacter baumanii(13%)

**Table 4. Percentage of different types of NFGNB**

| NFGNB | NO | % |
|---|---|---|
| *Pseudomonas aeruginosa* | 87 | 87 |
| *Acinetobacter baumanii* | 13 | 13 |
| Total | 100 | 100 |

**Figure 1. Percentage of different types of NFGNB**



## 5.4.Analysis by age and gender

### Distribution of NFGNB in different age group

Among the 100 isolates, maximum number of NFGNB were isolated from the agegroup of 40 – 50 in case of *Pseudomonas aeruginosa* (25.3%) . In case of *Acinetobacter baumanii* , patients more than 60 were more common (31%) followed by 40 – 50 year age group(23%).

**Table 5. Distribution of NFGNB in different age group**

| AGE | *PSEUDOMONAS AERUGINOSA* | | *ACINETOBACTER BAUMANII* | | TOTAL |
|---|---|---|---|---|---|
| | NO | % | NO | % | |
| <20 | 21 | 24 | 2 | 15.3 | 23 |
| 21 - 30 | 9 | 10.3 | 2 | 15.3 | 11 |
| 30 - 40 | 4 | 4.6 | 2 | 15.3 | 6 |
| 40 - 50 | 22 | 25.3 | 3 | 23 | 25 |
| 50 - 60 | 12 | 13.8 | - | - | 12 |
| >60 | 19 | 22 | 4 | 31 | 23 |
| TOTAL | 87 | 100 | 13 | 100 | 100 |

**Figure 2. Distribution of NFGNB in different age group**

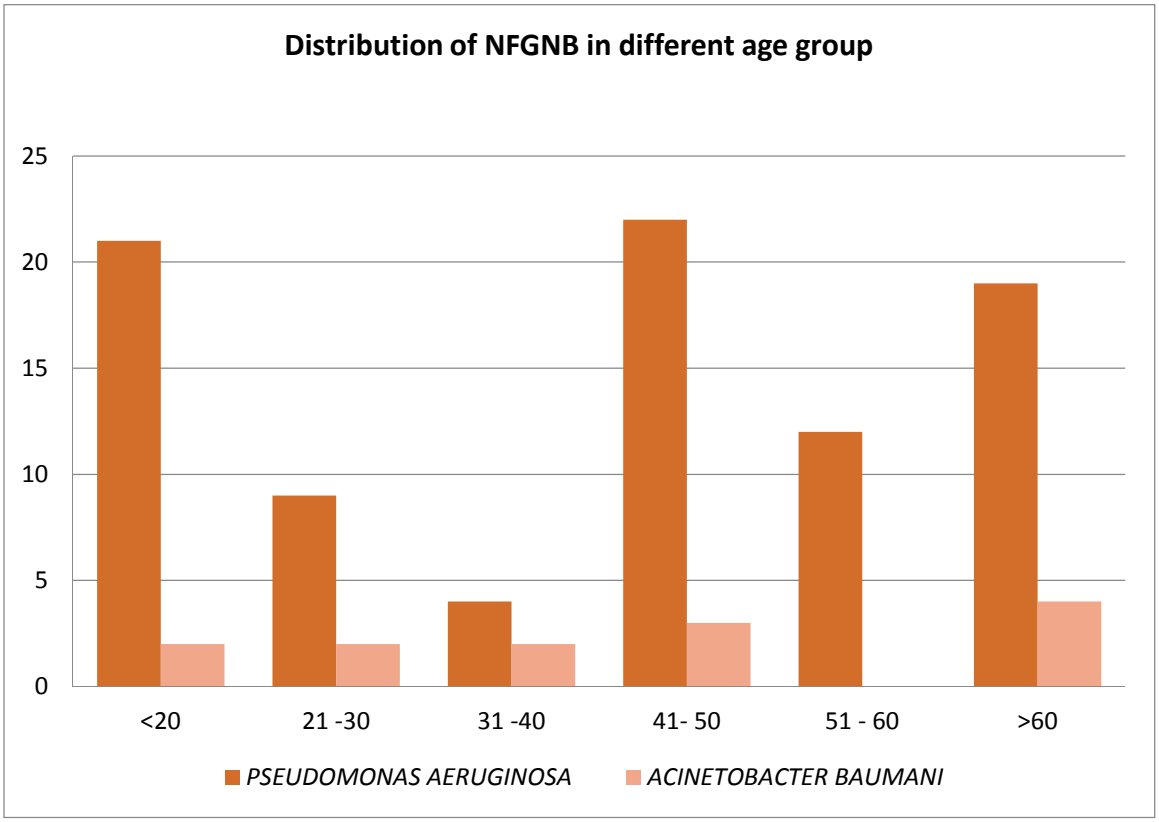



**Figure 3. Distribution of *P.aeruginosa* in different age group**

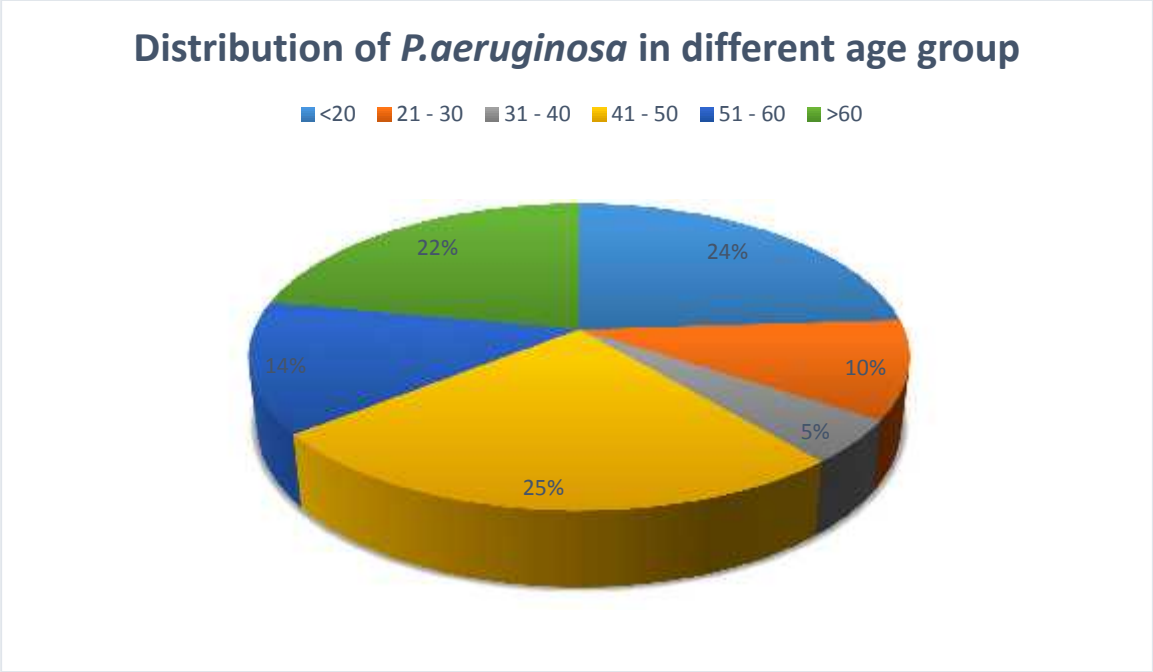
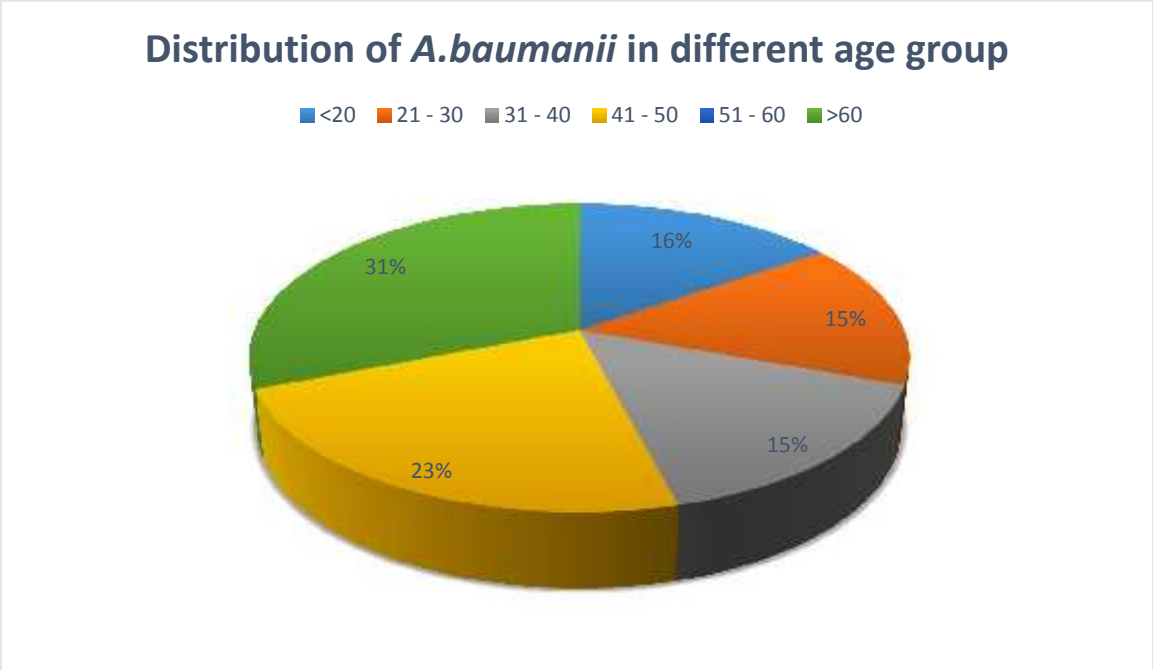



**Figure 4. Distribution of *A.bauamnii* in different age group**



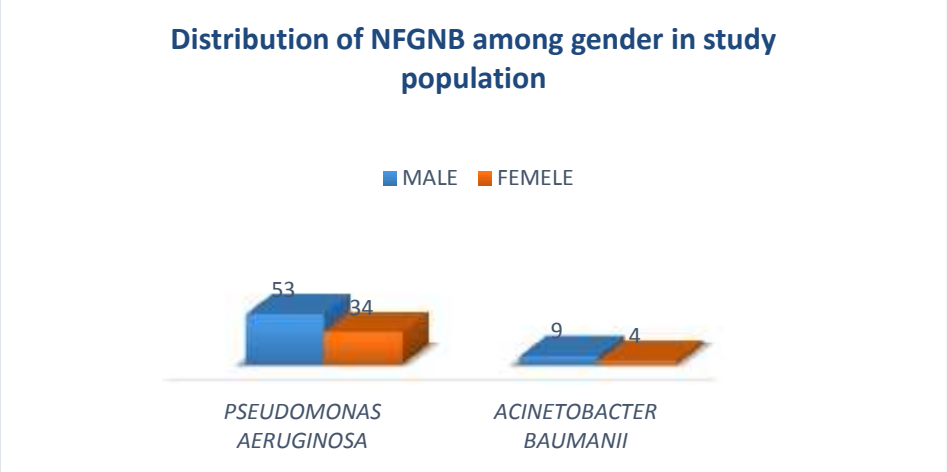
**Distribution of NFGNB among Gender in study population**

Male outnumbered female in case of rate of isolation of NFGNB. The sex ratio (male: female) for Pseudomonas aeruginosa was 1.4: 1and 2.25: 1 in case of Acinetobacter baumanii. (Table 6)

**Table 6. Distribution of NFGNB among Gender in study population**

| NFGNB | MALE | | FEMELE | | TOTAL |
|---|---|---|---|---|---|
| | NO | % | NO | % | |
| *PSEUDOMONAS AERUGINOSA* | 53 | 61 | 34 | 39 | 87 |
| *ACINETOBACTER BAUMANII* | 9 | 69 | 4 | 31 | 13 |
| TOTAL | 62 | | 38 | | 100 |

**Figure 5. Distribution of NFGNB among gender in study population**



## 5.5. Distribution of NFGNB in clinical samples

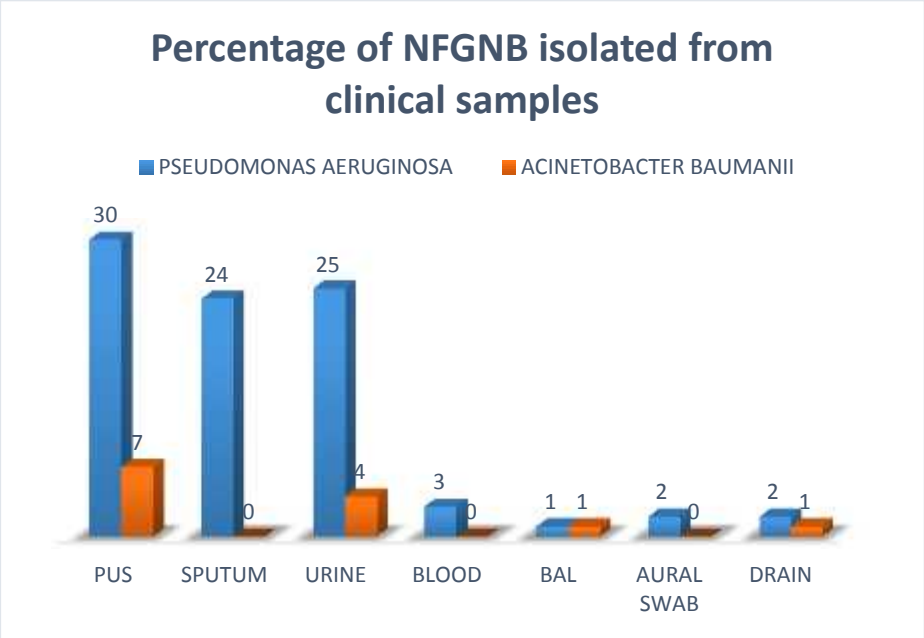
**Table 7**. P**ercentage of NFGNB isolated from clinical samples**

| SPECIMEN | *PSEUDOMONAS AERUGINOSA* | | *ACINETOBACTER BAUMANII* | | TOTAL |
|---|---|---|---|---|---|
| | NO | % | NO | % | |
| PUS | 30 | 34.5 | 7 | 54 | 37 |
| SPUTUM | 24 | 27.6 | - | - | 24 |
| URINE | 25 | 28.7 | 4 | 30.8 | 29 |
| BLOOD | 3 | 3.5 | - | - | 3 |
| BAL | 1 | 1.1 | 1 | 7.6 | 2 |
| AURAL SWAB | 2 | 2.3 | - | - | 2 |
| DRAIN | 2 | 2.3 | 1 | 7.6 | 3 |
| TOTAL | 87 | 100 | 13 | 100 | 100 |

From the above table it is inferred that 34.5% of *Pseudomonas aeruginosa* were isolated from the sample of pus, followed by 28.7% from urine, 27.6% from sputum, 3.5% from blood, 2.3% from aural swab and drain and 1.1% from BAL.

54% of *Acinetobacter baumanii* were isolated from pus, 30.8 % from urine and 7.65 from BAL and drain

**Figure 6**. **Percentage of NFGNB isolated from clinical samples**



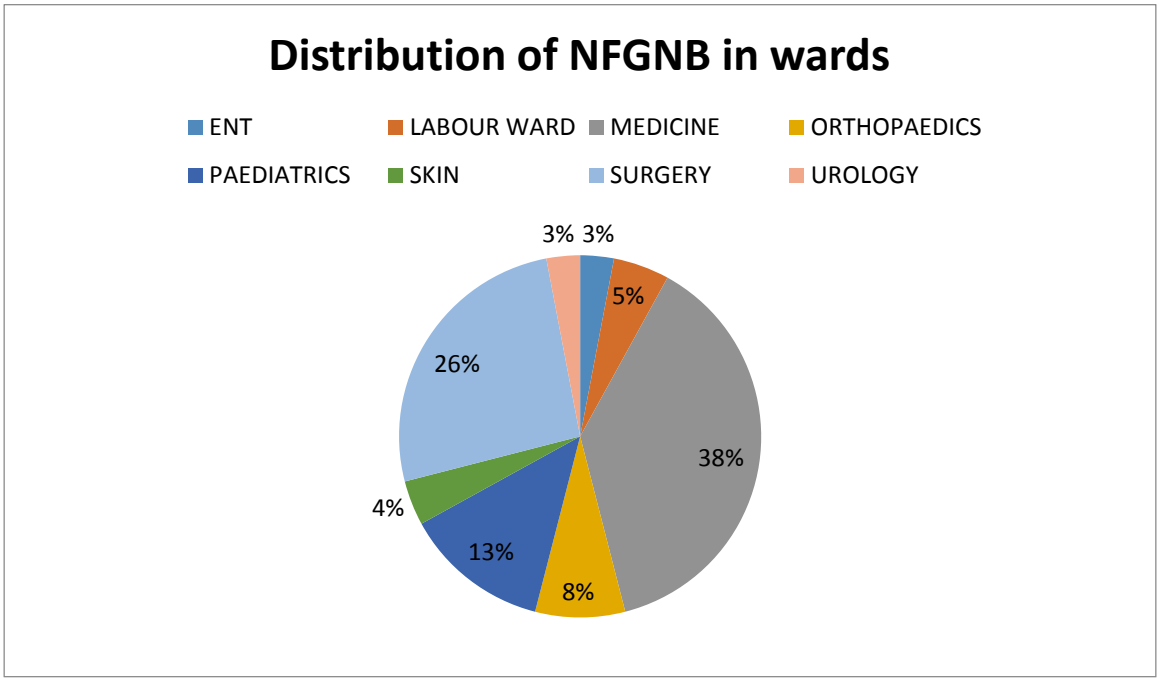
**Distribution of NFGNB in wards:**

**5.6.Table 8. Distribution of NFGNB in wards:**

| Ward | *Pseudomonas aeruginosa* | % | *Acinetobacter baumanii* | % | Total |
|---|---|---|---|---|---|
| ENT | 2 | 2.3 | 1 | 7.7 | 3 |
| LABOUR WARD | 4 | 4.6 | 1 | 7.7 | 5 |
| MEDICINE | 36 | 41.4 | 2 | 15 | 38 |
| ORTHOPAEDICS | 4 | 4.6 | 4 | 30.8 | 8 |
| PAEDIATRICS | 12 | 13.8 | 1 | 7.7 | 13 |
| SKIN | 4 | 4.6 | - | - | 4 |
| SURGERY | 23 | 26.4 | 3 | 23.4 | 26 |
| UROLOGY | 2 | 2.3 | 1 | 7.7 | 3 |
| **Total** | **87** | **100** | **13** | **100** | **100** |

From the above table, it was clear that *Pseudomonas aeruginosa* was isolated mainly from the Medicine wards (41.4%) followed by Surgery(26.4%) and Paediatrics(13.8%)wards. In case of *Acinetobacter baumanii* maximum isolation was from the Orthopaedics ward (30.8%) followed by Surgery ward(23.4%)

**Figure7. Distribution of NFGNB in wards**



### 5.7. Distribution of NFGNB in wards:

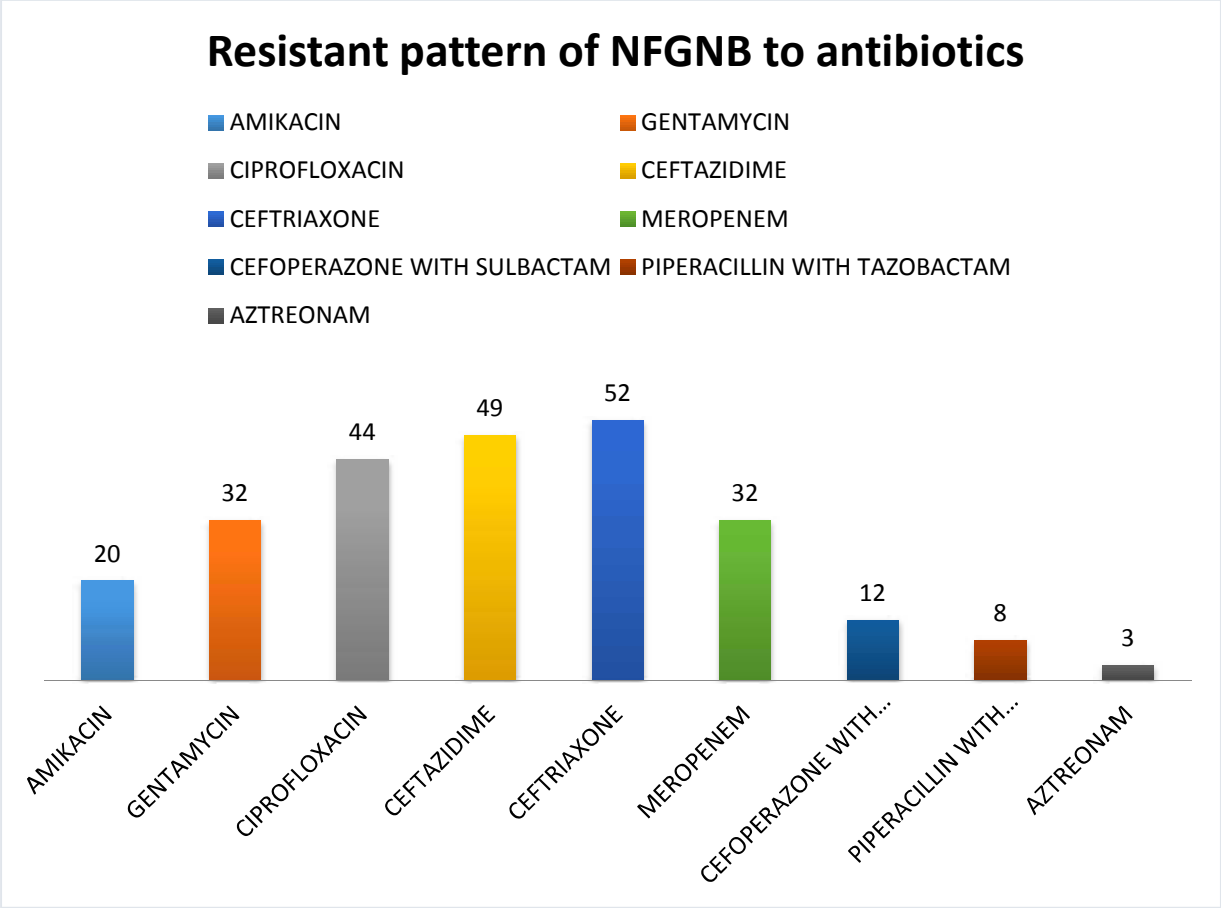
**Table 9. Resistant pattern of NFGNB to antibiotics**

| ANTIBIOTICS | NO OF RESISTANT STRAINS | % |
|---|---|---|
| AMIKACIN | 20 | 20 |
| GENTAMYCIN | 32 | 32 |
| CIPROFLOXACIN | 44 | 44 |
| CEFTAZIDIME | 49 | 49 |
| CEFTRIAXONE | 52 | 52 |
| MEROPENEM | 32 | 32 |
| CEFOPERAZONE WITH SULBACTAM | 12 | 12 |
| PIPERACILLIN WITH TAZOBACTAM | 8 | 8 |
| AZTREONAM | 3 | 3 |

The above table showed the resistant pattern to NFGNB . The isolated NFGNB showed maximum resistance to third generation cephalosporin such as ceftriaxone(52%), ceftazidime (49%)followed by ciprofloxacin(44%) and least resistance to aztreonam(3%). Piperacillin with tazobactam showed 8% resistance , cefoperazone with sulbactam showed 12% resistance. The rate of aminoglycoside resistance was less when compared to third

generation cephalosporins, amikacin and gentamycin showing 20% and 32% resistance respectively.

**Figure 8. Resistant pattern of NFGNB to antibiotics**



## 5.8. Meropenem resistance in NFGNB by disc diffusion test

**Table 10. Meropenem resistance in NFGNB by disc diffusion test**

| MEROPENNEM | PSEUDOMONAS AERUGINOSA | | ACINETOBACTER BAUMANII | | TOTAL |
|---|---|---|---|---|---|
| | NO | % | NO | % | |
| SENSITIVE | 62 | 71 | 6 | 46 | 68 |
| RESISTANT | 25 | 29 | 7 | 54 | 32 |
| TOTAL | 87 | 100 | 13 | 100 | 100 |

The above table showed the results for screening test for carbapenamase with meropenem disc. The test showed that 32% of the isolates were resistant to carbapenem. Among them, 25 isolates were *pseudomonas aeruginosa* and 7 isolates were for *Acinetobacter baumanii.*

**Figure 9. Meropenem resistance in NFGNB by disc diffusion test**

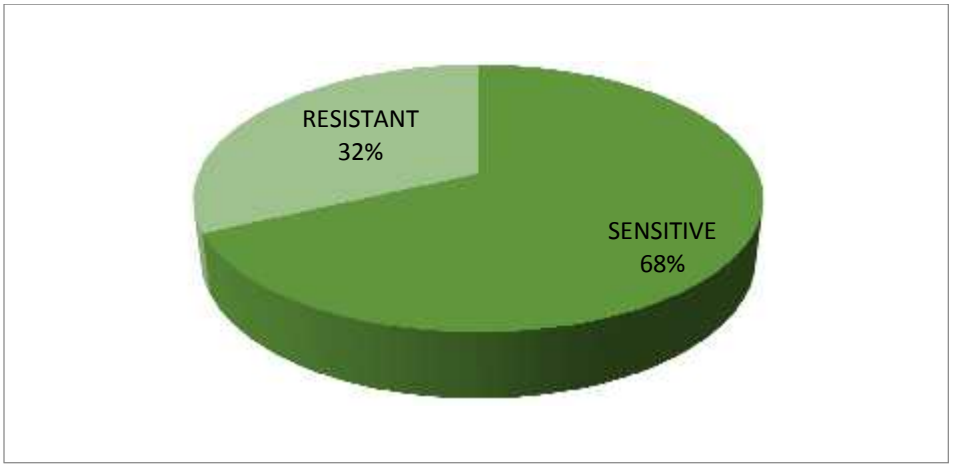
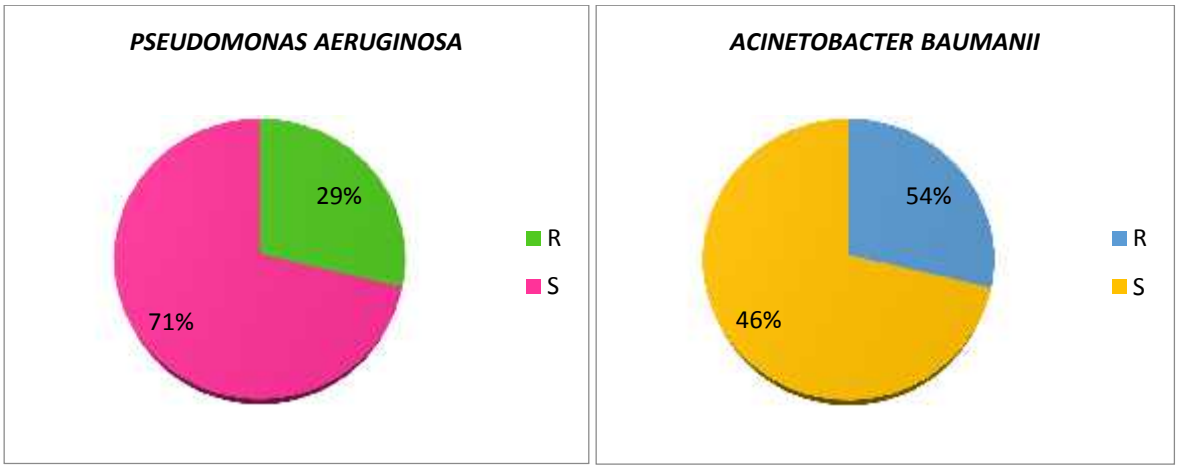


**Figure 10. Meropenem resistance *in Pseeudomonas aeruginosa* and *Acinetobacter baumanii* by disc diffusion test**



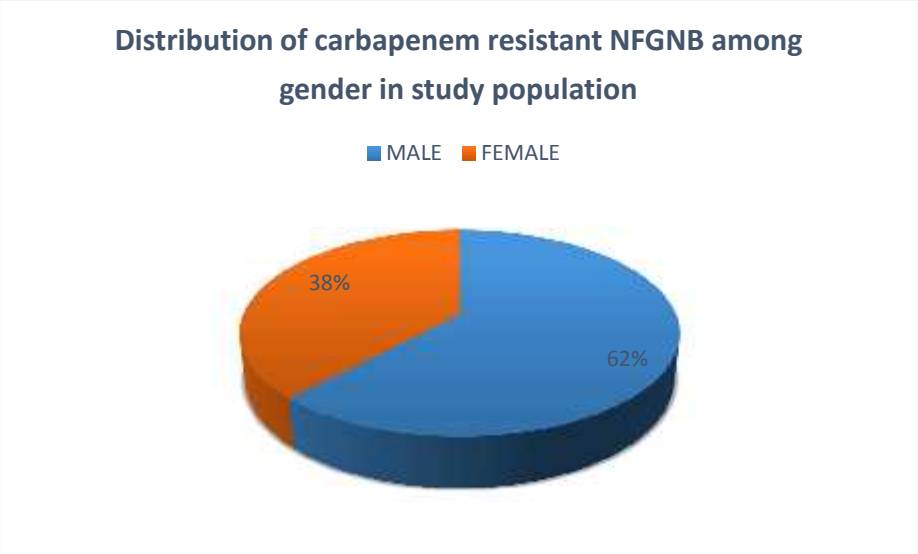
### 5.9. Distribution of carbapenem resistant NFGNB among gender in study population

**Table 11. Distribution of carbapenem resistant NFGNB among gender in study population**

| GENDER | CR NFGNB | % | CS NFGNB | % | TOTAL |
|---|---|---|---|---|---|
| MALE | 25 | 40.3 | 37 | 59.7 | 62 |
| FEMALE | 7 | 18.4 | 31 | 81.6 | 38 |
| TOTAL | 32 | | 68 | | 100 |

The above table shows gender distribution of carbapenem resistant NFGNB. Out of 32 resistant isolates,25 were from male and 7 were from female. The p value is 0.0226 and is statistically significant.

**Figure 11. Distribution of carbapenem resistant NFGNB among gender in study population**



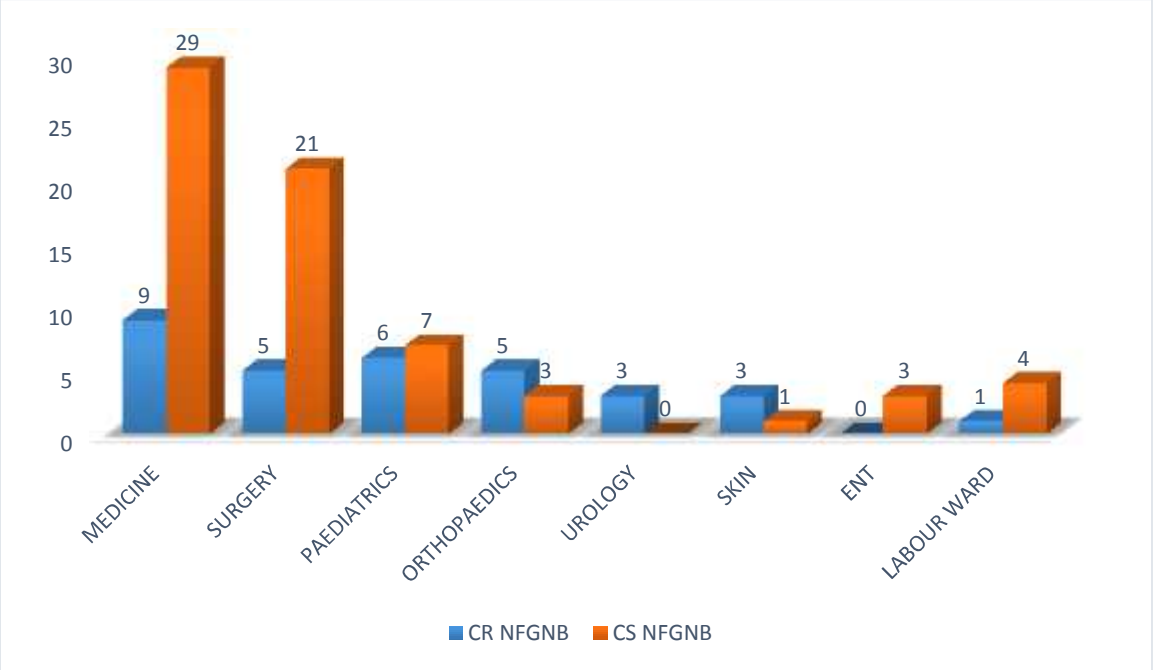
### 5.10. Distribution of CR NFGNB in wards

The table below shows the distribution of carbapenem resistant samples in various wards. Medical ward had maximum resistant isolates (28%) followed by Paediatrics and Surgery. ENT ward had no resistant isolate according to the present study.

**Table 12. Distribution of CR NFGNB in wards**

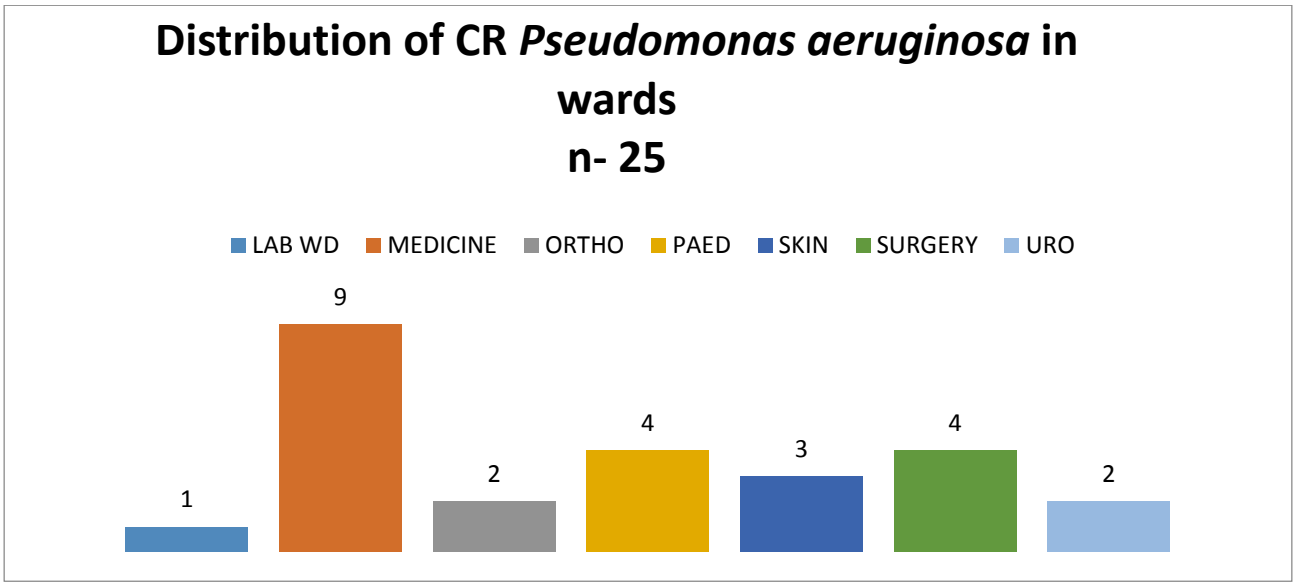
| WARD | CR NFGNB | | CS NFGNB | |
|---|---|---|---|---|
| | NO | % | NO | % |
| MEDICINE | 9 | 28 | 29 | 42.6 |
| SURGERY | 5 | 16 | 21 | 31 |
| PAEDIATRICS | 6 | 19 | 7 | 10.3 |
| ORTHOPAEDICS | 5 | 16 | 3 | 4.4 |
| UROLOGY | 3 | 9 | - | - |
| SKIN | 3 | 9 | 1 | 1.5 |
| ENT | - | - | 3 | 4.4 |
| LABOUR WARD | 1 | 3 | 4 | 5.8 |
| TOTAL | 32 | 100 | 68 | 100 |

**Figure 12. Distribution of CR NFGNB in wards**



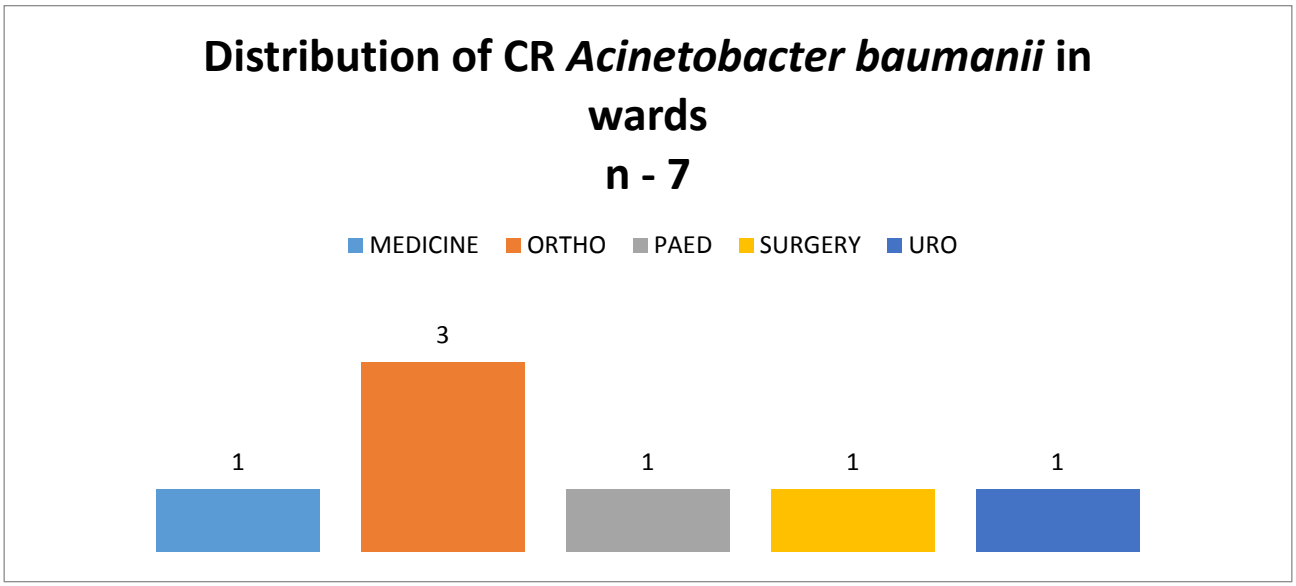

Maximum number of carbapenem resistant strains of Pseudomonas aeruginosa were isolated from medicine (9isolates) followed by Surgery and Paediatrics( 4 isolates each). Dermatology had 3 resistant isolate , Orthopaedics and Urology had 2 isolates each and Labour ward had 1 isolate

**Figure 13. Distribution of CR *pseudomonas aeruginosa* in wards**




Maximum number of carbapenem resistant strains of *Acinetobacter baumanii* were isolated from Orthopaedics (3isolates) followed by Surgery,Medicine , Urologyand Paediatrics(1 isolate each).

**Figure 14. Distribution of CRAcinetobacter baumanii in wards**




## 5.11.Distribution of CR NFGNB isolates in clinical samples

**Table 13. Distribution of CR NFGNB isolates in clinical samples**

The table below shows maximum number of CRNFGNB from of pus samples(40.6%), followed by urine (37.5%). The isolates from sputum showed 12.5 % resistance, blood and BAL showed 6.3% and 3.1 % respectively

| SPECIMEN | CR NFGNB | | CS NFGNB | |
|---|---|---|---|---|
| | NO | % | NO | % |
| PUS | 13 | 40.6 | 24 | 35.3 |
| SPUTUM | 4 | 12.5 | 20 | 29.4 |
| URINE | 12 | 37.5 | 17 | 25 |
| BLOOD | 2 | 6.3 | 1 | 1.5 |
| BAL | 1 | 3.1 | 1 | 1.5 |
| AURAL SWAB | - | - | 2 | 2.9 |
| DRAIN | - | - | 3 | 4.4 |
| TOTAL | 32 | 100 | 68 | 100 |

**Figure 15. Distribution of CR NFGNB isolates in clinical samples**

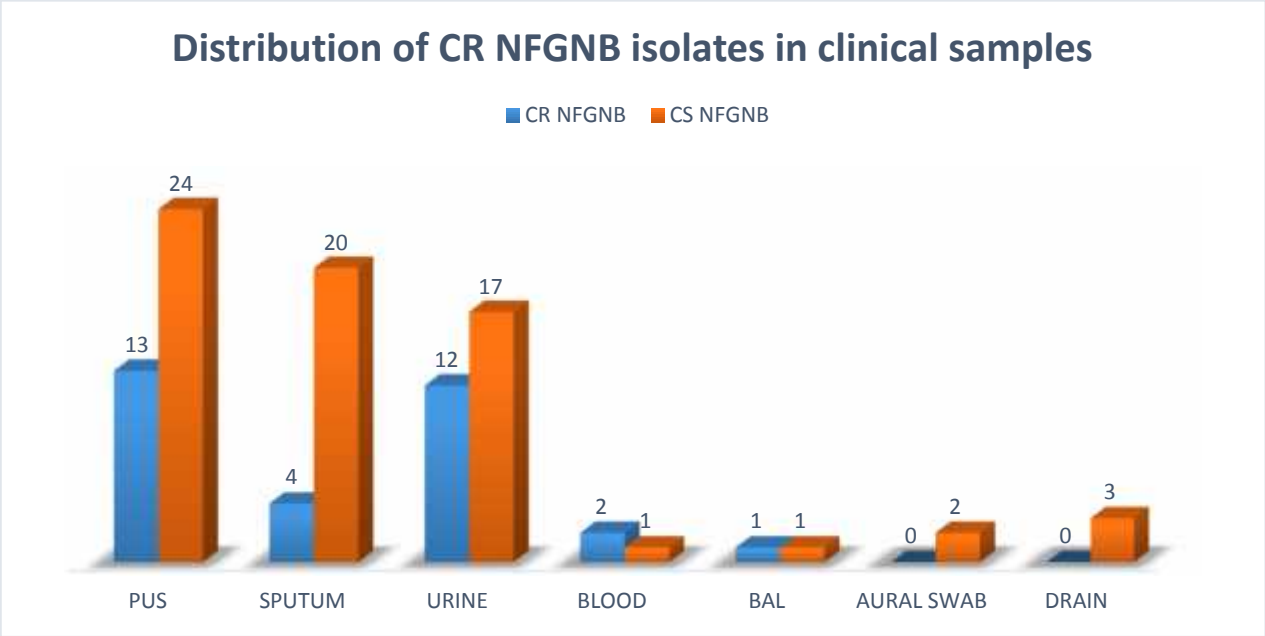
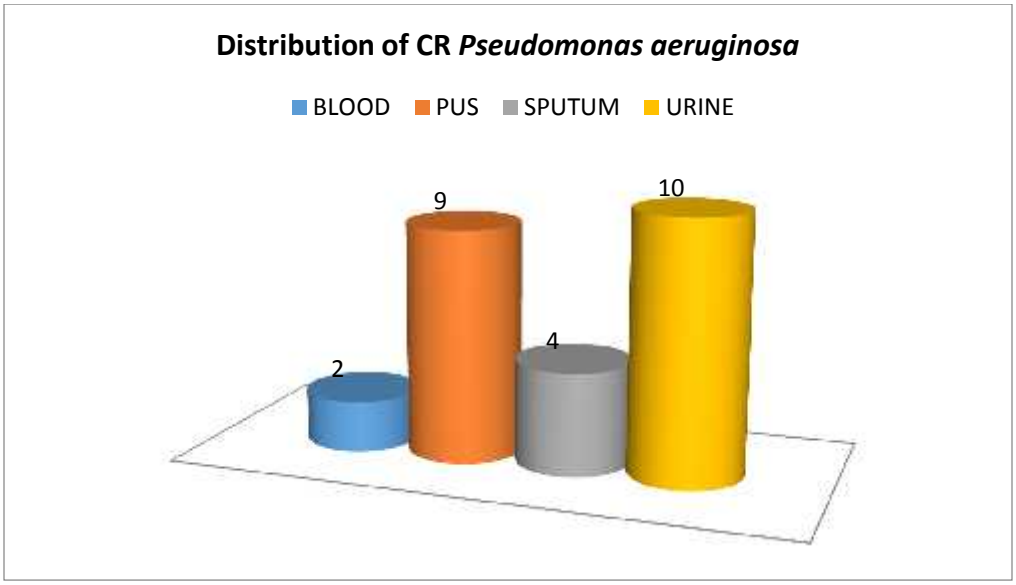
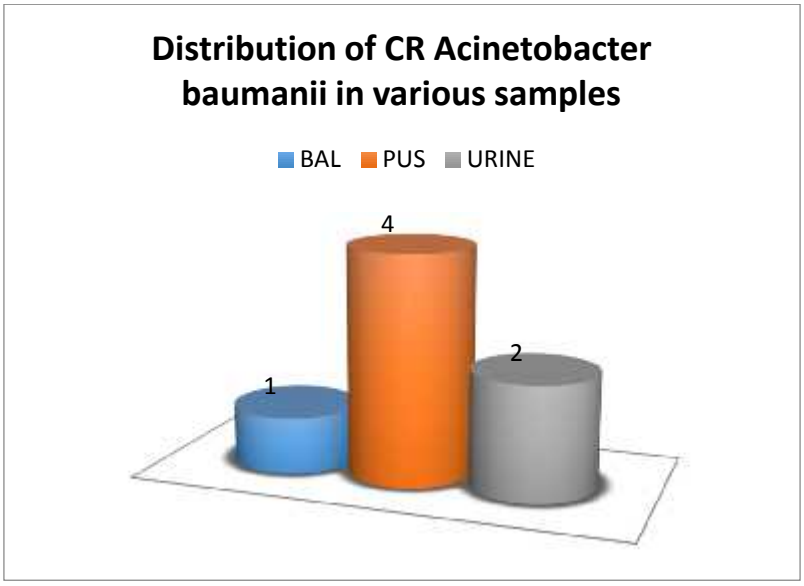



**Figure 16. Distribution of CR *Pseudomonas aeruginosa* in clinical samples**




Maximum number of CR resistant *Pseudomonas aeruginosa* were from urine, followed by pus, sputum and blood ( 10, 9, 4 and 2 respectively)

**Figure 17. Distribution of CR Acinetobacter baumanii in clinical samples**




Among 7 isolates of CR Acinetobacter baumanii, 4 were from pus , 2 from urineand 1 isolate was from BAL

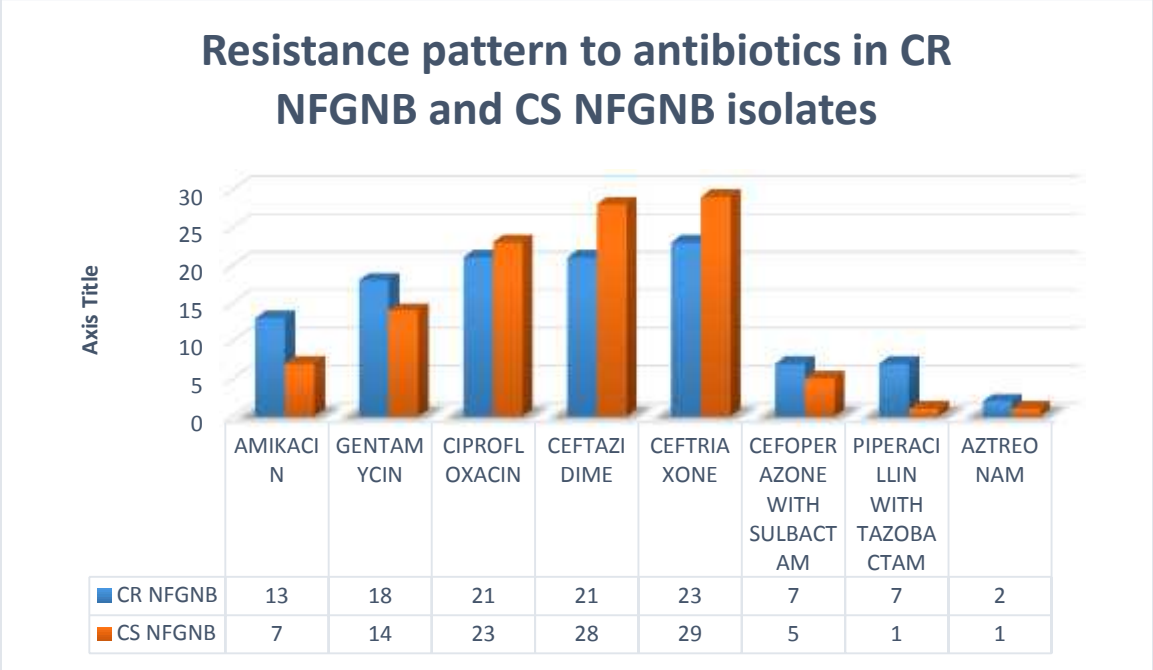
## 5.12. Resistance pattern to antibiotics in CR NFGNB and CS NFGNB isolates

**Table 14. Resistance pattern to antibiotics in CR NFGNB and CS NFGNB isolates**

| ANTIBIOTICS | CR NFGNB | | CS NFGNB | | TOTAL |
|---|---|---|---|---|---|
| | NO | % | NO | % | |
| AMIKACIN | 13 | 40.6 | 7 | 10.3 | 20 |
| GENTAMYCIN | 18 | 56.2 | 14 | 20.6 | 32 |
| CIPROFLOXACIN | 21 | 65.6 | 23 | 33.8 | 44 |
| CEFTAZIDIME | 21 | 65.6 | 28 | 41.1 | 49 |
| CEFTRIAXONE | 23 | 71.8 | 29 | 42.6 | 52 |
| CEFOPERAZONE WITH SULBACTAM | 7 | 21.8 | 5 | 7.3 | 12 |
| PIPERACILLIN WITH TAZOBACTAM | 7 | 21.8 | 1 | 1.4 | 8 |
| AZTREONAM | 2 | 6.3 | 1 | 1.4 | 3 |

There was statistically significant difference in exposure to cephalosporins among CR NFGNB and CS NFGNB isolates ($P<0.05$). There is no significance in the administration of other mentioned antibiotics with the corresponding CR NFGNB and CS NFGNB isolates.(Fig: 11)

**Figure 18. Resistance pattern to antibiotics in CR NFGNB and CS NFGNB isolates**




| | AMIKACIN | GENTAMYCIN | CIPROFLOXACIN | CEFTAZIDIME | CEFTRIAXONE | CEFOPERAZONE WITH SULBACTAM | PIPERACILLIN WITH TAZOBACTAM | AZTREONAM |
|---|---|---|---|---|---|---|---|---|
| CR NFGNB | 13 | 18 | 21 | 21 | 23 | 7 | 7 | 2 |
| CS NFGNB | 7 | 14 | 23 | 28 | 29 | 5 | 1 | 1 |

## 5.13. Detection of MBL by phenotypic methods

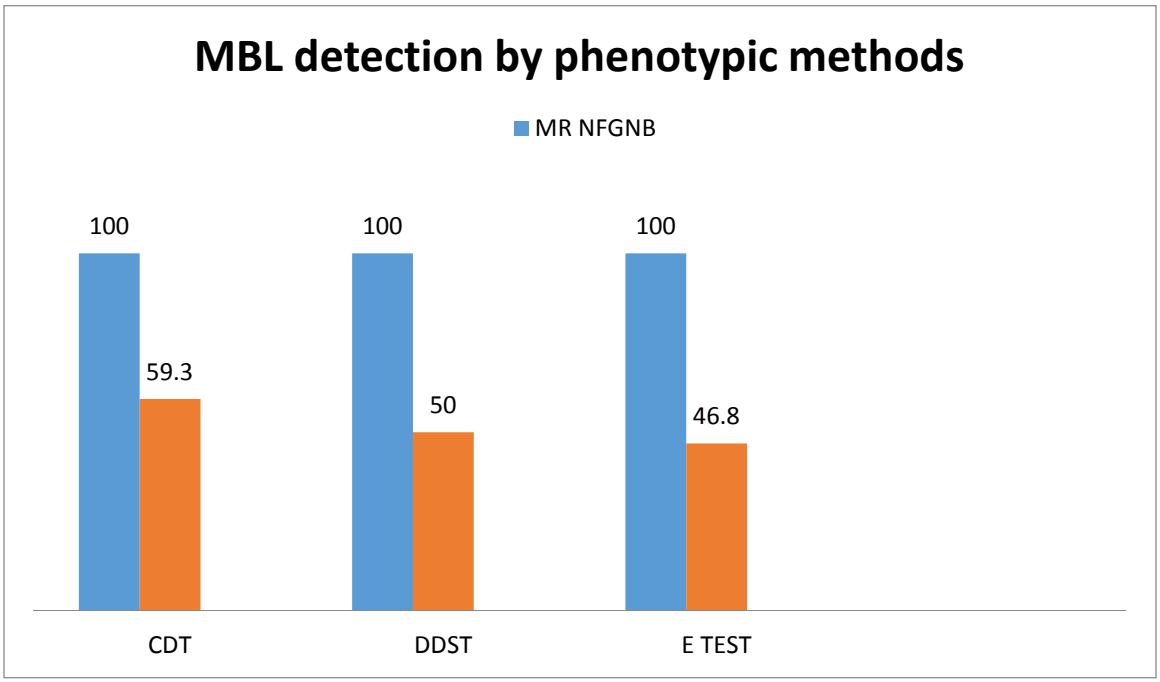
Out of 100 isolates, 32 showed meropenem resistance. They were subjected to phenotypic test for carbapenamase detection. CDT detected 19 isolates producing carbapenamse, DDST detected 16 isolates and E test detected 15 isolates producing carbapenamase.

CR NFGNB (n = 32)

**Table 15. Detection of MBL by phenotypic methods**

| METHOD | CR NFGNB | |
|---|---|---|
| | NO | % |
| CDT | 19 | 59.3 |
| DDST | 16 | 50 |
| E TEST | 15 | 46.8 |

**Figure 19. Detection of MBL by phenotypic methods**



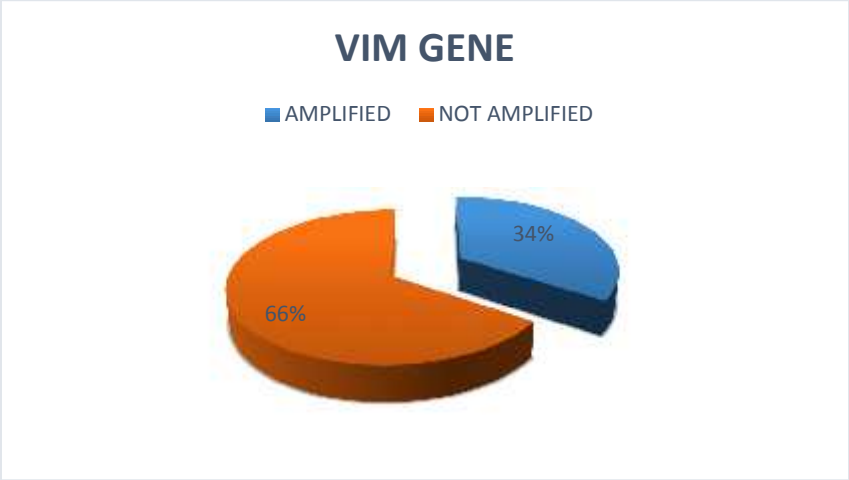

## 5.14. Detection of bla VIM gene by genotypic method

**Table 16. Detection of bla VIM gene by genotypic method**

| VIM GENE | NO OF ISOLATES | % |
|---|---|---|
| AMPLIFIED | 11 | 34.4 |
| NOT AMPLIFIED | 27 | 65.6 |

The above table showed the results of bla VIM detection by real time PCR. Out of 32 isolates, 11 isolates had bla VIM gene.

**Figure 20. Detection of bla VIM gene by genotypic method**




## 5.15. Comparision of CDT and PCR

**Table 17. Comparision of CDT and PCR**

| CDT | PCR | | TOTAL |
|---|---|---|---|
| | POSITIVE | NEGATIVE | |
| POSITIVE | 9 | 10 | 19 |
| NEGATIVE | 2 | 11 | 13 |
| TOTAL | 11 | 21 | 32 |

Among the 32 CR NFGNB, CDT detected 19 isolates with carbapenamase production, and PCR detects 11 isolates with carbapenamase production. When comparing

CDT and PCR, 9 isolates showed positivity by both PCR and CDT. 2 isolates which showed positivity in PCR were negative by CDT.

The sensitivity , specificity, positive predictive value, negative predictive value of CDT in the detection of carbapenamase were 81.8%, 52.3%, 47.3% and 84.6% respectively. The Kappa value denoting the degree of agreement is fair. (0.29)

### 5.16. Comparision of DDST and PCR

**Table 18. Comparision of DDST and PCR**

| DDST | PCR | | TOTAL |
|---|---|---|---|
| | POSITIVE | NEGATIVE | |
| POSITIVE | 9 | 7 | 16 |
| NEGATIVE | 2 | 14 | 16 |
| TOTAL | 11 | 21 | 32 |

Among the 32 CR NFGNB, DDST detected 16 isolates with carbapenamase production, and PCR detects 11 isolates with carbapenamase production. When comparing DDST and PCR, 9 isolates showed positivity by both PCR and DDST. 2 isolates which showed positivity in PCR were negative by DDST.

The sensitivity , specificity, positive predictive value, negative predictive value of DDST in the detection of carbapenamase were 81.8%, 66.7%, 56.2% and 87.5% respectively. The Kappa value denoting the degree of agreement is moderate (0.43)

## 5.17. Comparision of E TEST and PCR

**Table 19. Comparision of E TEST and PCR**

| E TEST | PCR | | TOTAL |
|---|---|---|---|
| | POSITIVE | NEGATIVE | |
| POSITIVE | 10 | 5 | 15 |
| NEGATIVE | 1 | 16 | 17 |
| TOTAL | 11 | 21 | 32 |

Among the 32 CR NFGNB, E test detected 15 isolates with carbapenamase production, and PCR detects 11 isolates with carbapenamase production. When comparing E test and PCR,10 isolates showed positivity by both PCR and E test. 1 isolate which showed positivity in PCR was negative by E test.

The sensitivity , specificity, positive predictive value, negative predictive value of E test in the detection of carbapenamase were 90.9%, 76.1%, 66.7% and 94% respectively. The Kappa value denoting the degree of agreement is good (0.61)

# 6. DISCUSSION

Non fermenter Gram negative bacilli are once considered as commensal and contaminant, but nowadays their pathological role is well established.[71] The outbreaks of nosocomial infections, emerging antimicrobial resistance and epidemiological complexity have made NFGNB the remarkable organism. They are now considered as important nosocomial pathogen.[68,69]Resistance pattern among the nosocomial bacterial pathogen may vary widely from place to place even within the same country over time.[70]

In the present study, out of 100 NFGNB , the most common organism isolated was *Pseudomonas aeruginosa* (87%) followed by *Acinetobacter baumanii* (13%). A similar observation was made by Vijaya et al 2009 which showed 78.9% *Pseudomonas spp* and 6.1 % *Acienetobacter spp*. A study conducted by Kamalraj et al also showed that among the non-fermenters, Pseudomonas spp.,(83.5%) were the maximum isolate followed by Acinetobacter spp(12.7).A study conducted by Gokhale and Metgud et al at 2013 at Banglore also showed the similar result with isolation rate of *Pseudomonas aerugin*osa 82.3% followed by 16% of *Acinetobacter baumanii*.

The present study showed that the maximum number of NFGNB were isolated from the age group of 40 – 50 in case of *Pseudomonas aeruginosa* (25.3%) . In case of *Acinetobacter baumanii* ,patients more than 60 were more commonly affected (31%) followed by 40 – 50 year age group(23%).Barnali Kakati et al at 2016 also found that there seems to be increasing trend of infections with NFGN bacilli maximum in 45-60 yrs

age group especially in medical patients with co-morbid illness[72,73] such as diabetes mellitus.

The sex ratio (male: female) for *Pseudomonas aeruginosa* was 1.4: 1and 2.25: 1 in case of *Acinetobacter baumanii* in this study. This is similar to study conducted by Ruchita Mahajan et al., in 2016 which showed 59.79% were isolated from males and 40.20% were from females.

Most of the *Pseudomonas aeruginosa* isolated in this study were from the sample of pus followed by urine and sputum . In case of *Acinetobacter baumanii* , maximum isolates were from pus followed by urine and BAL. A study by Mangaiarkkarasiet al [76] and Mahnaz saranghi et al[77] showed that *Pseudomonas aeruginosa* were mostly isolated from the pus samples (59-64%). This study indicates that the incidence of pyogenic infections is highest in the Medical and Surgical disciplines.

In the present study, *Pseudomonas aeruginosa* was isolated mainly from the Medical wards(41.4%) followed by Surgery(26.4%) and Paediatrics(13.8%)wards. In case of *Acinetobacter baumanii* maximum isolation was from the Orthopaedics ward (30.8%) followed by Surgery ward(23.4%). This observation is in concordance with Maniyan G et al which showed maximum number of isolates were from Surgical wards (28.18%) [79] .

The present study showed maximum resistance to third generation cephalosporin such as ceftriaxone(52%), ceftazidime (49%)followed by ciprofloxacin(44%) and least resistance to aztreonam(3%). Combination drugs with β lactam inhibitor such ass Piperacillin with tazobactam showed 8% resistance , cefoperazone with sulbactam showed

12% resistance. Similar results also observed in other studies with 60-70% resistance to ceftazidime and ciprofloxacin.[80]Many authors like Vikas kumar et al, Yousef Irfani et al[82] and Mahnaz et al have reported 52-70% of resistance towards cephalosporins in their study. Benachinmardi, *et al* showed 80% sensitivity to amikacin which is parallel to the present study which showed 20% resistant strains to amikacin and 32% to gentamycin.

Meropenem is used to detect carbapenam resistance in the current study according to CLSI guidelines. Screening test for carbapenem resistance was done with meropenem disc by disc diffusion method. In the present study 32% of the isolates showed meropenem resistance .Among them 29.5% of *P.aeruginosa* showed resistance to meropenem, while it was 54% in case of *Acinetobacter baumanii.* This showed that A. bauamnii is more resistant. Ruta S Patwardhan's study also agrees with the present study and showed 31.46 %resistance to Meropenem. A study by Manoharan et al has shown 42.7 % resistance to Meropenem. .

In India, resistance to carbapenems in *A. baumannii* ranges from 14% to 59%. Most resistant isolates were recovered from respiratory samples of patients in the ICU. [Gladstone et al 2005,Taneja et al.2003,Mahajan et al.2011, Noyal et al. 2009,Gupta 126 et al.2006, Wattal et al. 2010]

Carbapenem resistance in *P. aeruginosa* has been reported from centres in Pondicherry, Vellore, Bangalore, Chandigarh, Mumbai, New Delhi, and Varanasi with the rates of resistance between 10.9% and 69%. [Shashikala et al.2006,Taneja et al. 2003, Noyal et al. 2009, Navneeth et al. 2002, Behera et al.2008, Kumar et al.2011,Varaiya et al.2008, Wattal et al.2010, Kaul et al.2007]. In a multicentric study including centres all

over India conducted during 2005-07, 42.6 % of P. aeruginosa were resistant to imipenem/meropenem. [Manoharan et al.2010].

Meropenem resistant strains were screened for MBL production by CDT, IMP-EDTA disc synergy test and MBL E test (Behera et al.,[84] 2008; Lee et al., 2003; Arakawa et al., 2000[83]).

The present study detected 59.8% and 50% MBL producers by CDT and DDST among meropenem resistant NFGNB respectively.

Studies across India report rates of MBL production ranging from 72%-100% among carbapenem resistant NFGNB. The first report of MBL production in India was in 2002 from urban hospital in Bangalore which reported MBL production in all the carbapenem resistant *P.aeruginosa*. In 2005 Hemalatha V et al reported MBL production among 87.5% of carbapenem resistant *P.aeruginosa*. From which is similar to the findings in our study. Gupta V et al 21 reported MBL production in 86.11% of imepenem resistant NFGNB using imepenem EDTA-DDST method. Varaiya et al 22 recorded MBL production in 83.3% carbapenem resistant P.aeruginosa isolated from ICU patients with 100% resistance to aminoglycosides and cephalosporins.

A number of studies were conducted in various parts of the world for MBL detection which showed increased sensitivity to DDST compared to CDT. But in our study CDT was superior to DDST in MBL detection which is in concordance with Behera et al. This discrepancy may be due to the difference in geographical regions, difference in kind of infections, the vast usage of antimicrobials, or difference in antibiotic policy in the particular hospitals.[85,86,87]

There was 48.3% positivity for MBL E test in our study among the meropenem resistant isolates. Manoharan et al in their study reported 42.6% positive MBL producers by E test[88] which is in agreement with the present study results. Walsh TR, et al. reported 63.5% positive results[89]In contrast to the present study, Bashir et al showed higher prevalence of MBL of about 86% of carbapenem resistant isolates were positive by the E test[90].anonther study also showed 100% sensitivity for MBL E test disseminated multi-drug resistance in *Acinetobacter* and its counteracting mechanisms occurring in the recent scenario, compared to the earlier times.[91,92]

Eleven out of 32 isolates were positive for bla VIM gene among the carbapenem resistant isolates in the present study.The prevalence of MBL in India has ranged from 7% to 65% among carbapenem-resistant *P. aeruginosa*. [Arunagiri et al.2012, Manoharan et al. 2010]. In one study, the rate of MBL production was 24.5% among 61 *P. aeruginosa* isolates, and bla VIM type was the most common [Manoharan et al. 2010]. Another study from India also reported bla VIM-2 from P. aeruginosa [Toleman et al. 2007]. In a nationwide survey conducted to characterise 301 MBL producing *Pseudomonas* species in 10 medical centres from India, MBL genes were detected in 18.9% of the isolates and 5 VIM variants were reported with VIM-2 being the most common. The others were VIM-6, VIM-11, VIM-5 and VIM-18. [Castanheira et al 2009]. In India, MBL production among *A. baumannii* isolates has been reported as 42%. There is limited data on the prevalence and distribution of MBLs in *A. baumannii* among Indian isolates. . In an another study by Mlynarczyk et al showed that the PCR detected 60% of the carbapenem resistant isolates

as MBL producer and the gene isolated was *bla*VIM and it accounted for about 70% of the resistant isolates[92]

The present study compared phenotypic methods such as CDT, DDST, MBL E test with the genotypic methods such as detection of bla VIM gene by real time PCR.

The sensitivity and specificity of CDT were 81.8% and 52.3% respectively when compared to PCR for MBL detection. The present study is in agreement with the study conducted by Manoharan et al that showed the sensitivity and specificity as 87.8% and 53.3%, respectively when compared to the PCR[93]. But CDT showed 100% sensitivity and 96% specificity in a study conducted by Johann D. D. Pitout et al.[94]

DDST when compared to PCR showed the sensitivity and specificity of 81.8% and 66.7% in the present study. reported the The sensitivity of the DDST was 85.7% in a study done by Bachunde et al, in Maharashtra, 2011 which correlates with the present study[95]. S John et al and Yalda khosravi et al in their study reported higher sensitivity and specificity for DDST when compared to CDT[96,97]

Among the carbapenem resistant isolates, MBL E test showed 90.9% sensitivity and 76% specificity when compared to PCR. Yalda kosravi et al in their study reported that the E test has given the sensitivity and specificity of 100% which does not correlates with the current study[97]. Kyungon Lee et al in their study reported that the MBL E test is highly sensitive and specific for detecting *bla*IMP and *bla*VIM allele positive isolates of *P.aeruginosa* .

Out of the 32 carbapenem resistant isolates, CDT and DDST detected 19 1nd 16 MBL producers. The kappa value denoting the degree of agreement is also fair and

moderate. This denotes that phenotypic test were not efficient in detecting the MBL as PCR. The same result has been reported in Franco MR et al[98] Brazil and Fereshteh Shahcheraghi et al[99].Those isolates which showed positive by phenotypic test and not by PCR were considered as false positive. Despite the good performance of inhibitor based methods for the detection of MBL by using EDTA, false positive results have been reported in P. aeruginosa as EDTA acts on the membrane of the bacterial cell and increase the cell permeability.

The sensitivity and specificity of MBL e test was 90% and 76% respectively. The kappa value denoting the degree of agreement was also good. Hence this test can be used to detect MBL in case of non availability of genotypic methods. But the cost factor of this test limits its routine laboratory usage in resource poor settings.

Not all Meropenem resistant isolates were found to be MBL producersin the present study. Alexandre R. Marra et al, Ami Varaiya et al, Chacko et al, Ting ting Qu et al also showed the similar results in their study. Other mechanisms of such as mutation in the outer membrane permeability, loss of porins or the upregulation of efflux systems attributed to the resistance. Jose Manuel Rodriguez Martínez et al[100], Elena Riera et a[101], O. Gutierrez, C et al [102]have done studies on these mechanism.

Among the CR NFGNB, cephalosporins showed 65-70% resistance and ciprofloxacin showed 65% resistance. This is due to the wide usage of these antibiotics in the community in the treatment of suspected Gram negative infections.

About 40-56% of the CR NFGNB isolates were resistant to amikacin and gentamicin. Fereshteh Shahcheraghi et al also reported that most MBL producers were

resistant to aminoglycosides. Since aminoglycosides were the widely used antimicrobial agents in association with other antibiotics might be the cause for the resistance.

Among the 32 CR NFGNB, 13 were multidrug resistant non fermenters. MBL producers were 5 among them. All of them were susceptible to aztreonam.

. Drug resistance also leads to  epidemics, and consequently there is a greater risk of infection to others. Improved infection control measures and judicious antibiotic usage are necessary to contain the emergence and spread of multiply drug resistant nonfermenters in health care setting. Moreover, timely dissemination of the local antibiogram will aid the clinician in choosing the appropriate antibiotic.

## 7. SUMMARY

In the present study,

- 87% of the NFGNB isolates were *Pseudomonas aeruginosa* and 13% were *Acinetobacter baumanii*.
- Maximum number of NFGNB were isolated from the age group of 40 – 50 in case of *Pseudomonas aeruginosa* (25.3%) . In case of *Acinetobacter baumanii* ,patients more than 60 were more common (31%) followed by 40 – 50 year age group(23%).
- The gender distribution ratio (male: female) for *Pseudomonas aerugin*osa was 1.4: 1and 2.25: 1 in case of *Acinetobacter baumanii*.
- 34.5% of *Pseudomonas aeruginosa* were isolated from the sample of pus, followed by 28.7% from urine, 27.6% from sputum , 3.5% from blood, 2.3% from aural swab and drain and 1.1% from BAL. In case of 54% of *Acinetobacter baumanii* were isolated from pus, 30.8 % from urine and 7.65 from BAL and drain
- *Pseudomonas aeruginosa* was isolated mainly from the Medicine wards(41.4%) followed by Surgery(26.4%) and Paediatrics(13.8%)wards. In case of *Acinetobacter baumanii* maximum isolation was from the Orthopaedics ward (30.8%) followed by Surgery ward(23.4%)
- The isolated NFGNB showed maximum resistance to third generation cephalosporin such as ceftriaxone(52%), ceftazidime (49%)followed by ciprofloxacin(44%) and least resistance to aztreonam(3%).
- Screening test for carbapenamase with meropenem disc by disc diffusion test showed that 32% of the isolates were resistant to carbapenem. Among them, 25

isolates were resistant for *pseudomonas aeruginosa* and 7 isolates were resistant for *Acinetobacter baumanii*.

- Gender distribution of carbapenem resistant NFGNB showed 25 isolates were from male and 7 isolates were from female. Among the 7 CR resistant *Acinetobacter baumanii*, 5 were from male and 2 isolates were from female.
- Medical ward had maximum resistant isolates (28%) followed by Paediatrics and Surgery. ENT ward had no resistant isolate.
- Maximum number of CRNFGNB from the sample of pus (40.6%), followed by urine (37.5%). Sputum showed 12.5 % resistance, blood and BAL showed 6.3% and 3.1 % respectively.
- CDT detected 59.3% , DDST 50% and E test 46.8% of MBL producer among the 32 meropenem resistant NFGNB
- The sensitivity , specificity, positive predictive value, negative predictive value of CDT in the detection of carbapenamase were 81.8%, 52.3%, 47.3% and 84.6% respectively.
- The sensitivity , specificity, positive predictive value, negative predictive value of DDST in the detection of carbapenamase were 81.8%, 66.7%, 56.2% and 87.5% respectively.
- The sensitivity , specificity, positive predictive value, negative predictive value of E test in the detection of carbapenamase were 90.9%, 76.1%, 66.7% and 94% respectively.
- 11 isolates were positive for bla VIM gene by real time PCR.

- Out of 32 meropenem resistant isolates, 13 were multidrug resistant non fermenters and MBL producers were 5 among them. All of them were susceptible to aztreonam.

# 8. CONCLUSION

This study deals with the clincoepidemiological profile of non fermenter Gram negative bacilli among the clinical specimens received in the laboratory and also prevalence of bla VIM gene among the CR NFGNB.

Though NFGNB are regarded as contaminant, they are responsible for wide range of nosocomial infections. These organisms can also spread resistance to other susceptible bacteria by horizontal gene transfer. Variability in sensitivity pattern emphasizes the need for identification of NFGNB and to monitor their susceptibility patterns as it helps in proper and effective management of the infection caused by them.

In view of carbapenem resistance and MDR isolates, there is a need to emphasize on strict adherence to the concept of “reserve drugs” and antibiotic therapy should be advocated or modified following culture and sensitivity. This will help in the proper treatment of the patient and will discourage the indiscriminate use of available antibiotics and curtail the spread of drug resistant bacteria. Moreover, considering the prevalence of carbapenem resistant bacteria, it is necessary to carry out regular monitoring of drug resistance and molecular characterization of carbapenem resistant isolates.

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## ANNEXURE– 1

**1.Nutrient agar medium:**

**Composition**

**Ingredients gram/liter**

Peptic digest of Animal Tissue 5.00

Sodium Chloride 5.00

Beef Extract 1.50

Yeast Extract 1.50

Agar 15.00

Twenty-eight grams of dehydrated nutrient agar medium was added to 1000 ml ofcold distilled water in a flask and boiled to dissolve the medium completely. Themedium was then sterilized in an autoclave at $121^0$C and 15 lbs pressure for 15minutes. The sterile media were stored in a refrigerator at $4^0$C for future use.

**2. MacConkey agar medium:**

Composition - Ingredients gram/liter

Peptone 19.0

Lactose 10.0

NaCl 5.0

Na- Deoxycholate 1.0

Neutral Red 0.03

Crystal Violet 0.001

Agar 15.0

Fifty-two grams of dehydrated MacConkey agar medium was suspended in 1000 ml of cold distilled water and boiled to dissolve the medium completely. The solution wasthen sterilized by autoclaving at $121^0$C and 15 lbs pressure for 15 minutes.

**3. Blood agar medium**

**Composition**

**Ingredients gram/liter**

Heart infusion 500.00

Tryptose 10.00

Sodium chloride 5.00

Agar 15.00

Forty grams of the dehydrated blood agar medium was suspended in 1000 ml colddistilled water in a flask and boiled to dissolve the medium completely. It was thensterilized by autoclaving at $121^0$C and 15 lbs pressure for 15 minutes. The autoclavedmaterials were allowed to cool to a temperature of $45^0$C in a water bath. Defibrinated5-10% sheep blood was then added to the medium aseptically and distributed tosterile petridishes. Sterile media was stored in refrigerator at $4^0$C for future use.

**4.Triple sugar agar medium**

| **Composition** | **Ingredients Gms / Litre** |
|---|---|
| Beef extract | 3.000 |
| Peptone | 20.000 |
| Yeast extract | 3.000 |

| | |
|---|---|
| Lactose | 10.000 |
| Sucrose | 10.000 |
| Dextrose monohydrate | 1.000 |
| Ferrous sulphate | 0.200 |
| Sodium chloride | 5.000 |
| Sodium thiosulphate | 0.300 |
| Phenol red | 0.024 |
| Agar | |

Suspend 64.42 grams of dehydrated medium in 1000 ml distilled water. Heat to boiling. Dissolve the medium completely. Mix well and distribute into test tubes and Sterilize by maintaining at 10lbs pressure ($115^0$C) for 30 minutes. Allow the medium to set in sloped form with a butt about 2.5cm long.

**5. Muller Hinton agar medium**

Composition

| Ingredients | gram/liter |
|---|---|
| Beef dehytrated infusion | 300 |
| Casein hydrolysate | 17.50 |
| Starch agar | 17.00 |
| Agar | 17.00 |

Thirty-eight grams of dehydrated Mueller Hinton agar medium was suspended in1000 ml cold distilled water and boiled to dissolve the medium completely. Thesolution

was then sterilized by autoclaving at $121^0$C and 15 lbs pressure for 15minutes. The autoclaved media was stored in the refrigerator and used later.

**6. McFarland Standard (0.5):**

**Reagents:**

Sulphuric acid,1%: To 100 ml of distilled water,1 ml of conc.sulphuric acid is added.Barium chloride, 1.175%: To 100 ml of distilled water, 1.175gm of barium chloride is added and mixed well.

**To prepare McFarland 0.5 standards:**

To 85 ml of 1% conc.sulphuric acid, 0.5 ml of Barium chloride is added in a flask while constantly swirling the flask. Bring to 100 ml with 1% conc.sulphuric acid. Aliquot in test tubes and cap tubes tightly. Store in the dark at room temperature for 3 months or longer.

## ANNEXURE– II

## PCR REQUIREMENTS:

- Bacterial pellet
- Micro Pipettes variable volume 0.5-10 µl, 10-100 µl, and 100-1000 µl
- Sterile tips.
- Vortex mixer
- Water bath
- 13,000 rpm Centrifuge (Refrigerated) with rotor for 1.5ml reaction tubes
- 1.5ml/2ml centrifuge tubes
- Thermo cycler (Biorad CFX 96)
- Computer for data storage

column. The isolated DNA can be used directly for the PCR amplification.

**Components of DNA extraction**

- Phosphate buffered saline
- Lysozyme
- Digestion buffer
- Binding buffer
- Proteinase K
- Internal control template
- Isopropanol
- 70% Ethanol
- Elution buffer

**ANNEXURE– III**

**PROFORMA**

Name :

Age :

Sex :

OP/IP No :

Lab No :

Ward :

Complaints :

Clinical diagnosis :

Nature of Specimen :

Duration of hospital stay :

Antibiotics administered :

Investigation :

Biochemical tests : Indole, Citrate, Urease, Triple sugar iron,

Catalase, Oxidase

Antibiogram :

Piperacillin, Amikacin, Ceftriaxone, Cefotaxime, Ceftazidime, Ciprofloxacin, ,Gentamicin, Meropenem, Piperacillin with Tazobactam, Cefoperazone and sulbactam, aztreonam

Screening test with meropenam

Combined disc test

Double disc synergy test

MBL E test

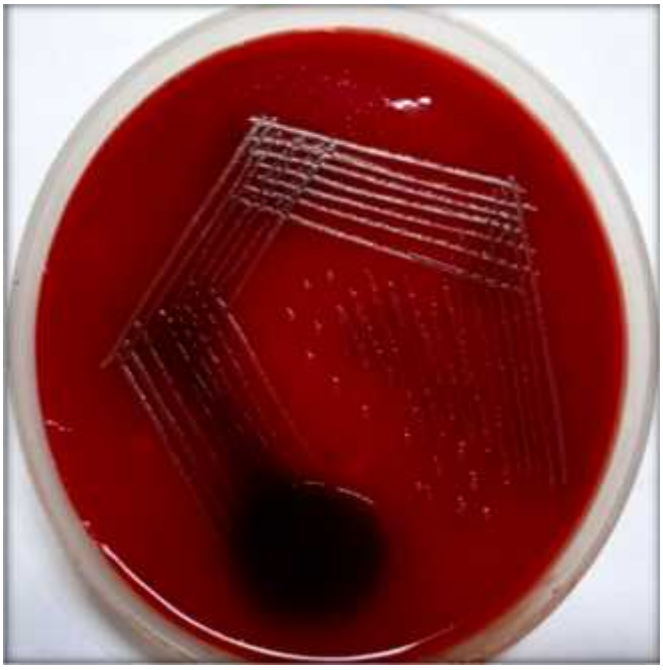
RT-PCR

## ANNEXURE– V

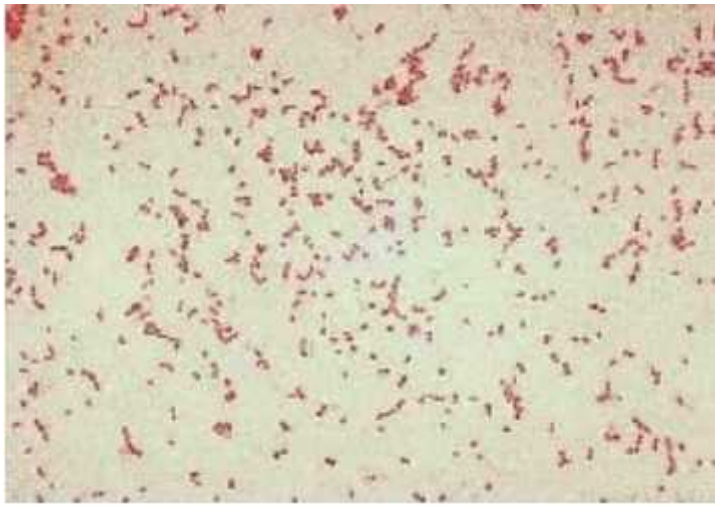
## NUTRIENT AGAR PLATE SHOWING GREEN PIGMENTED COLONIES

## MACKONKEY AGAR PLATE SHOWING NON-LACTOSE FERMENTING COLONIES

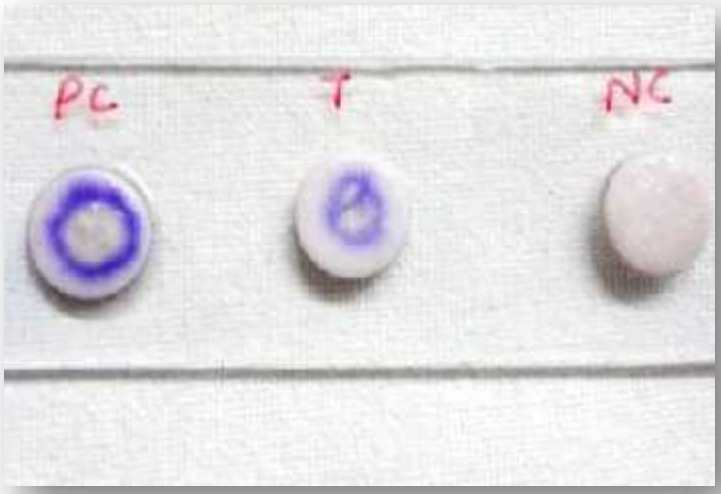
## BLOOD AGAR PLATE SHOWING METALLIC SHEEN AND BETA HEMOLYTIC COLONIES



## ACINETOBACTER BAUMANII - GRAM STAIN



## OXIDASE TEST – DRY FILTER PAPER METHOD




## BIOCHEMICAL REACTIONS OF P.AERUGINOSA

## BIOCHEMICAL REACTIONS OF A.BAUAMNII


## ANTIBIOTIC SUSCEPTIBILITY TEST

# COMBINED DISC TEST

## MBL Producer


## Non MBL Producer

**DOUBLE DISC SYNERGY TEST**
**THE ENHANCEMENT OF THE ZONE OF INHIBITION FROM MEROPENEM DISC TOWARDS EDTA DISC**

**MBL producer by DDST**

**MBL E Test**

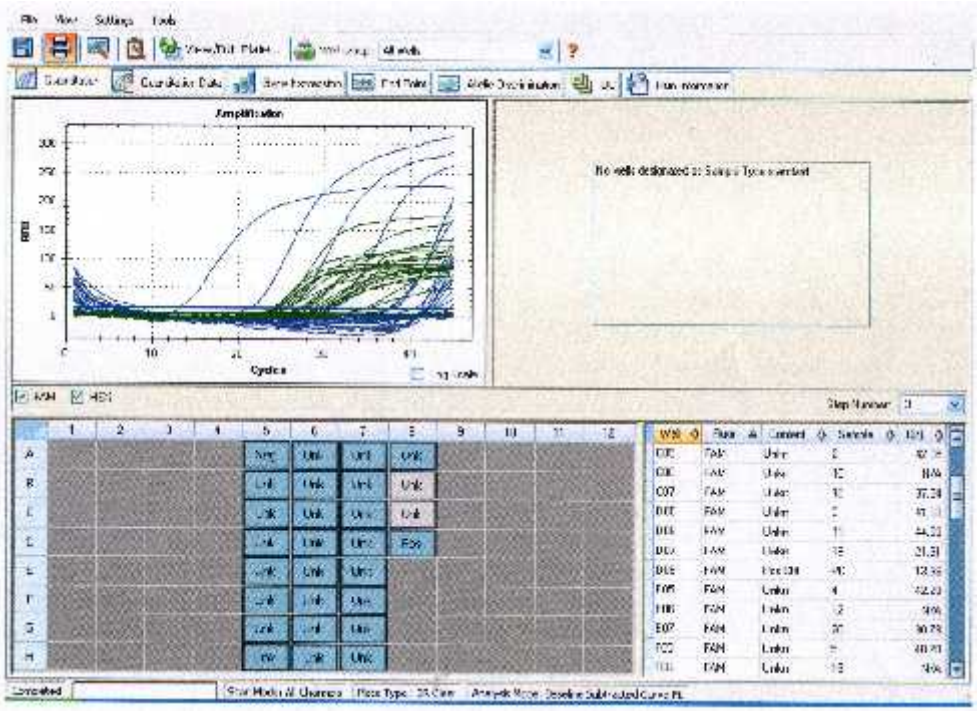
**MBL-PRODUCER:**


**NON-MBL PRODUCER:**

# MOLECULAR CHARACTERISATION by PCR: THERMOCYCLER

## DNA Extraction Kit:

## PCR – RESULT



| S. NO | LAB NO | AGE | SEX | WARD | DIAGNOSIS | SPECIMEN | NFGNB | AMIKACIN | GENTAMYCIN | CIPROFLOXACIN | COTRIMOXAZOLE | CEFTAIZIDIME | CEFTRIAXONE | MEROPENEM | CEFOPERAZONE SULBACTAM | PIPERACILLIN TAZOBACTAM | AZTREONAM | DDST | CDT | E TEST |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 4347 | 2 | F | PAED | UTI | URINE | PSEUDO. AEURO | R | R | R | R | R | R | S | R | S | S | | | |
| 2 | 4344 | 3 | M | PAED | UTI | URINE | PSEUDO. AEURO | S | S | S | R | R | R | S | R | S | S | | | |
| 3 | 4444 | 11 | F | PAED | UTI | URINE | PSEUDO. AEURO | S | S | S | R | S | S | R | S | S | S | N | N | N |
| 4 | 4016 | 65 | M | SURGERY | WOUND INF | PUS | PSEUDO. AEURO | R | R | R | R | R | R | R | S | S | S | N | N | N |
| 5 | 3990 | 18 | F | MEDICINE | PNEUMONIA | SPUTUM | PSEUDO. AEURO | S | S | R | R | R | R | S | S | S | S | | | |
| 6 | 3926 | 42 | M | SURGERY | ABSCESS | PUS | PSEUDO. AEURO | S | S | S | R | R | R | S | S | S | S | | | |
| 7 | 4412 | 70 | M | URO | PYELO | URINE | PSEUDO. AEURO | R | R | R | R | R | R | R | S | S | S | P | P | P |
| 8 | 4247 | 2 | F | PAED | UTI | URINE | PSEUDO. AEURO | S | S | S | R | S | S | S | S | S | S | | | |
| 9 | 4242 | 65 | F | MEDICINE | UTI | URINE | PSEUDO. AEURO | R | R | R | R | S | S | R | S | S | S | N | N | N |
| 10 | 3828 | 69 | M | MEDICINE | PNEUMONIA | SPUTUM | PSEUDO. AEURO | S | S | S | R | S | S | S | S | S | S | | | |
| 11 | 3918 | 85 | M | MEDICINE | PNEUMONIA | SPUTUM | PSEUDO. AEURO | S | S | S | R | R | R | S | S | | S | | | |
| 12 | 3920 | 43 | F | SURGERY | ABSCESS | PUS | PSEUDO. AEURO | S | S | S | R | R | R | S | S | S | S | | | |
| 13 | 4223 | 28 | F | LAB WD | UTI | URINE | PSEUDO. AEURO | S | S | R | R | S | R | S | S | S | S | | | |
| 14 | 4293 | 67 | M | MEDICINE | UTI | URINE | PSEUDO. AEURO | S | S | R | R | R | R | R | S | R | R | N | P | N |

| S. NO | LAB NO | AGE | SEX | WARD | DIAGNOSIS | SPECIMEN | NFGNB | AMIKACIN | GENTAMYCIN | CIPROFLOXACIN | COTRIMOXAZOLE | CEFTAIZIDIME | CEFTRIAXONE | MEROPENEM | CEFOPERAZONE SULBACTAM | PIPERACILLIN TAZOBACTAM | AZTREONAM | DDST | CDT | E TEST |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15 | 3901 | 62 | F | SURGERY | WOUND INF | PUS | PSEUDO. AEURO | R | S | S | s | R | R | R | S | R | R | P | P | P |
| 16 | 3887 | 62 | F | MEDICINE | PNEUMONIA | SPUTUM | PSEUDO. AEURO | S | S | S | R | S | S | S | S | S | S | | | |
| 17 | 3894 | 42 | M | SURGERY | WOUND INF | DRAIN | PSEUDO. AEURO | S | S | S | R | S | S | S | S | S | S | | | |
| 18 | 4248 | 2 | F | PAED | UTI | URINE | PSEUDO. AEURO | R | R | R | R | R | R | R | S | S | S | P | P | P |
| 19 | 3880 | 29 | F | MEDICINE | PNEUMONIA | BAL | PSEUDO. AEURO | S | S | S | S | R | R | R | R | R | S | N | P | N |
| 20 | 3881 | 65 | M | ORTHO | WOUND INF | PUS | PSEUDO. AEURO | S | R | S | S | R | R | R | R | S | S | P | P | P |
| 21 | 3861 | 45 | M | MEDICINE | PNEUMONIA | SPUTUM | PSEUDO. AEURO | S | S | S | R | R | R | S | S | S | S | | | |
| 22 | 3858 | 69 | F | MEDICINE | PNEUMONIA | SPUTUM | PSEUDO. AEURO | S | S | S | R | R | R | S | S | S | S | | | |
| 23 | 3984 | 11 | M | SURGERY | WOUND INF | PUS | PSEUDO. AEURO | S | S | S | R | S | S | S | S | S | S | | | |
| 24 | 3888 | 45 | F | MEDICINE | PNEUMONIA | SPUTUM | PSEUDO. AEURO | S | S | S | R | S | S | S | S | S | S | | | |
| 25 | 3981 | 58 | M | SURGERY | ABSCESS | PUS | PSEUDO. AEURO | S | S | R | R | R | R | S | S | S | S | | | |
| 26 | 3967 | 55 | M | SURGERY | WOUND INF | PUS | PSEUDO. AEURO | S | R | R | R | R | R | S | S | S | S | | | |
| 27 | 3829 | 60 | M | SURGERY | ABSCESS | PUS | PSEUDO. AEURO | S | R | R | R | R | R | R | S | R | S | N | N | N |
| 28 | 3694 | 30 | M | ENT | CSOM | AURAL SWAB | PSEUDO. AEURO | S | S | S | R | R | R | S | S | S | S | | | |

| S. NO | LAB NO | AGE | SEX | WARD | DIAGNOSIS | SPECIMEN | NFGNB | AMIKACIN | GENTAMYCIN | CIPROFLOXACIN | COTRIMOXAZOLE | CEFTAIZIDIME | CEFTRIAXONE | MEROPENEM | CEFOPERAZONE SULBACTAM | PIPERACILLIN TAZOBACTAM | AZTREONAM | DDST | CDT | E TEST |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 29 | 3682 | 17 | F | MEDICINE | PNEUMONIA | SPUTUM | PSEUDO. AEURO | S | S | S | R | R | R | R | S | S | S | N | N | N |
| 30 | 3683 | 70 | F | MEDICINE | PNEUMONIA | SPUTUM | PSEUDO. AEURO | S | S | S | R | S | S | S | S | S | S | | | |
| 31 | 4163 | 60 | M | SURGERY | ABSCESS | PUS | PSEUDO. AEURO | R | R | R | R | S | S | S | S | S | S | | | |
| 32 | 4149 | 51 | M | ORTHO | WOUND INF | PUS | PSEUDO. AEURO | R | R | R | R | R | R | R | R | S | S | P | P | P |
| 33 | 4143 | 60 | M | MEDICINE | PNEUMONIA | SPUTUM | PSEUDO. AEURO | S | S | S | R | S | S | S | S | S | S | | | |
| 34 | 4145 | 50 | M | SURGERY | ABSCESS | PUS | PSEUDO. AEURO | R | R | S | R | S | S | S | S | S | S | | | |
| 35 | 4545 | 28 | M | MEDICINE | PNEUMONIA | SPUTUM | PSEUDO. AEURO | S | S | R | R | S | S | R | S | S | S | N | P | P |
| 36 | 4093 | 55 | M | MEDICINE | PNEUMONIA | SPUTUM | PSEUDO. AEURO | S | S | S | R | S | S | S | S | S | S | | | |
| 37 | 4514 | 18 | F | MEDICINE | PNEUMONIA | SPUTUM | PSEUDO. AEURO | S | S | R | R | S | S | S | S | S | S | | | |
| 38 | 4077 | 38 | M | SURGERY | ABSCESS | PUS | PSEUDO. AEURO | R | R | R | R | R | R | R | S | S | S | N | P | P |
| 39 | 4522 | 60 | F | MEDICINE | UTI | URINE | PSEUDO. AEURO | S | S | S | S | S | S | S | S | S | S | | | |
| 40 | 4499 | 31 | F | LAB WD | UTI | URINE | PSEUDO. AEURO | S | S | S | S | S | S | S | S | S | S | | | |
| 41 | 4501 | 8 | M | PAED | UTI | URINE | PSEUDO. AEURO | S | S | S | S | S | S | S | S | S | S | | | |
| 42 | 4595 | 12 | F | PAED | UTI | URINE | PSEUDO. AEURO | S | S | S | S | S | S | S | S | S | S | | | |

| S. NO | LAB NO | AGE | SEX | WARD | DIAGNOSIS | SPECIMEN | NFGNB | AMIKACIN | GENTAMYCIN | CIPROFLOXACIN | COTRIMOXAZOLE | CEFTAIZIDIME | CEFTRIAXONE | MEROPENEM | CEFOPERAZONE SULBACTAM | PIPERACILLIN TAZOBACTAM | AZTREONAM | DDST | CDT | E TEST |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 43 | 4545 | 45 | M | MEDICINE | UTI | URINE | PSEUDO. AEURO | S | S | R | R | R | R | R | R | S | S | N | P | P |
| 44 | 4198 | 58 | F | SURGERY | WOUND INF | PUS | PSEUDO. AEURO | S | S | S | R | S | S | S | S | S | S | | | |
| 45 | 4576 | 18 | M | SKIN | ABSCESS | PUS | PSEUDO. AEURO | R | R | R | R | R | R | R | R | S | S | P | P | N |
| 46 | 4649 | 18 | M | SKIN | ABSCESS | PUS | PSEUDO. AEURO | S | S | R | R | R | R | R | S | S | S | P | P | N |
| 47 | 5242 | 55 | M | MEDICINE | UTI | URINE | PSEUDO. AEURO | S | S | S | S | S | R | R | S | S | S | P | P | P |
| 48 | 4716 | 45 | M | MEDICINE | LRTI | SPUTUM | PSEUDO. AEURO | S | S | S | S | S | R | R | S | S | S | P | P | P |
| 49 | 4638 | 41 | M | SURGERY | ABSCESS | PUS | PSEUDO. AEURO | S | R | R | R | R | R | S | S | S | S | | | |
| 50 | 5148 | 29 | F | LAB WD | UTI | URINE | PSEUDO. AEURO | S | R | R | R | R | R | S | S | S | S | | | |
| 51 | 4997 | 45 | M | MEDICINE | UTI | URINE | PSEUDO. AEURO | S | S | S | R | R | R | S | S | S | S | | | |
| 52 | 4982 | 25 | F | LAB WD | UTI | URINE | PSEUDO. AEURO | R | R | R | R | S | S | R | S | R | S | P | P | P |
| 53 | 4497 | 45 | M | SURGERY | WOUND INF | DRAIN | PSEUDO. AEURO | S | S | R | R | R | S | S | S | S | S | | | |
| 54 | 4510 | 50 | M | SURGERY | WOUND INF | PUS | PSEUDO. AEURO | S | S | S | R | S | S | S | S | S | S | | | |
| 55 | 5160 | 45 | M | MEDICINE | UTI | URINE | PSEUDO. AEURO | S | S | R | R | R | R | S | S | S | S | | | |
| 56 | 5135 | 45 | M | MEDICINE | UTI | URINE | PSEUDO. AEURO | S | S | S | R | R | R | S | S | S | S | | | |

| S. NO | LAB NO | AGE | SEX | WARD | DIAGNOSIS | SPECIMEN | NFGNB | AMIKACIN | GENTAMYCIN | CIPROFLOXACIN | COTRIMOXAZOLE | CEFTAIZIDIME | CEFTRIAXONE | MEROPENEM | CEFOPERAZONE SULBACTAM | PIPERACILLIN TAZOBACTAM | AZTREONAM | DDST | CDT | E TEST |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 57 | 4529 | 57 | M | MEDICINE | PNEUMONIA | SPUTUM | PSEUDO. AEURO | S | R | R | R | S | S | S | S | S | S | | | |
| 58 | 4510 | 45 | M | SURGERY | WOUND INF | PUS | PSEUDO. AEURO | S | S | S | R | R | R | S | S | S | S | | | |
| 59 | 4502 | 66 | M | MEDICINE | PNEUMONIA | SPUTUM | PSEUDO. AEURO | S | S | S | R | S | S | R | S | S | S | P | P | P |
| 60 | 4397 | 47 | M | ORTHO | WOUND INF | PUS | ACINETO | S | S | R | R | S | R | R | R | S | S | P | N | N |
| 61 | 4466 | 42 | F | MEDICINE | PNEUMONIA | SPUTUM | PSEUDO.A EURO | R | S | S | R | S | R | S | S | S | S | | | |
| 62 | 4402 | 63 | M | ENT | CSOM | AURAL SWAB | PSEUDO.A EURO | S | S | S | R | S | S | S | S | S | S | | | |
| 63 | 4599 | 48 | F | SURGERY | HAEMOTHORAX | SPUTUM | PSEUDO.A EURO | S | S | S | R | R | R | S | R | S | S | | | |
| 64 | 4487 | 45 | M | SURGERY | WOUND INF | DRAIN | PSEUDO.A EURO | S | S | R | R | S | S | S | S | S | S | | | |
| 65 | 1513 | 3 | F | PAED | SEPSIS | BLOOD | PSEUDO. AEURO | S | S | S | R | S | S | S | S | S | S | | | |
| 66 | 4338 | 70 | F | ORTHO | WOUND INF | PUS | PSEUDO. AEURO | S | S | R | R | S | S | S | S | S | S | | | |
| 67 | 4355 | 43 | M | SURGERY | ABSCESS | PUS | PSEUDO. AEURO | S | S | S | R | S | S | S | S | S | S | | | |
| 68 | 4336 | 48 | F | SURGERY | WOUND INF | PUS | PSEUDO. AEURO | S | R | R | R | S | R | S | S | S | S | | | |
| 69 | 4363 | 46 | F | MEDICINE | PNEUMONIA | BAL | PSEUDO. AEURO | S | S | S | R | S | R | S | S | S | S | | | |
| 70 | 4916 | 54 | M | SKIN | ABSCESS | PUS | PSEUDO. AEURO | S | S | S | R | S | S | S | S | S | S | | | |
| 71 | 5849 | 33 | M | MEDICINE | UTI | URINE | ACINETO | S | S | S | S | R | S | S | S | S | S | | | |

| S. NO | LAB NO | AGE | SEX | WARD | DIAGNOSIS | SPECIMEN | NFGNB | AMIKACIN | GENTAMYCIN | CIPROFLOXACIN | COTRIMOXAZOLE | CEFTAIZIDIME | CEFTRIAXONE | MEROPENEM | CEFOPERAZONE SULBACTAM | PIPERACILLIN TAZOBACTAM | AZTREONAM | DDST | CDT | E TEST |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 72 | 5499 | 27 | M | MEDICINE | UTI | URINE | PSEUDO. AEURO | S | S | R | R | S | R | R | S | S | S | N | P | P |
| 73 | 4922 | 29 | F | SURGERY | ABSCESS | PUS | PSEUDO. AEURO | S | S | S | R | R | R | S | S | S | S | | | |
| 74 | 5549 | 45 | F | LAB WD | UTI | URINE | ACINETO | S | S | R | R | R | S | S | S | S | S | | | |
| 75 | 4882 | 66 | M | SURGERY | ABSCESS | PUS | PSEUDO. AEURO | R | R | R | R | S | S | R | S | S | S | P | N | P |
| 76 | 5439 | 65 | M | URO | UTI | URINE | ACINETO | R | R | R | R | R | R | R | S | R | S | N | N | N |
| 77 | 1640 | 5 | M | PAED | SEPSIS | BLOOD | PSEUDO. AEURO | R | R | S | R | S | S | R | S | S | S | P | N | N |
| 78 | 4905 | 25 | F | MEDICINE | PYOTHORAX | PUS | PSEUDO. AEURO | R | R | R | R | R | S | S | S | S | S | | | |
| 79 | 4898 | 58 | F | MEDICINE | PNEUMONIA | SPUTUM | PSEUDO. AEURO | S | R | S | R | S | S | S | S | S | S | | | |
| 80 | 5462 | 11 | M | PAED | UTI | URINE | ACINETO | S | R | R | R | R | S | R | R | S | S | P | P | P |
| 81 | 4777 | 47 | M | ORTHO | ABSCESS | PUS | PSEUDO. AEURO | R | R | R | R | R | R | S | S | S | S | | | |
| 82 | 4785 | 50 | M | SKIN | ABSCESS | PUS | PSEUDO. AEURO | S | S | S | R | R | S | R | S | S | S | P | P | N |
| 83 | 4186 | 15 | F | SURGERY | WOUND INF | PUS | PSEUDO. AEURO | S | S | S | R | S | S | S | S | S | S | | | |
| 84 | 4183 | 72 | F | MEDICINE | PNEUMONIA | SPUTUM | PSEUDO. AEURO | R | S | S | R | S | S | S | S | S | S | | | |
| 85 | 1705 | 2 | M | PAED | SEPSIS | BLOOD | PSEUDO. AEURO | R | R | R | R | R | R | R | S | S | S | N | N | N |
| 86 | 5008 | 42 | M | ORTHO | WOUND INF | PUS | PSEUDO. AEURO | S | R | R | R | R | R | R | S | R | S | N | N | N |
| 87 | 5548 | 38 | M | ORTHO | WOUND INF | PUS | ACINETO | S | R | R | R | R | R | R | S | S | S | N | N | N |

| S. NO | LAB NO | AGE | SEX | WARD | DIAGNOSIS | SPECIMEN | NFGNB | AMIKACIN | GENTAMYCIN | CIPROFLOXACIN | COTRIMOXAZOLE | CEFTAIZIDIME | CEFTRIAXONE | MEROPENEM | CEFOPERAZONE SULBACTAM | PIPERACILLIN TAZOBACTAM | AZTREONAM | DDST | CDT | E TEST |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 88 | 4717 | 6 | M | PAED | UTI | URINE | PSEUDO. AEURO | S | R | R | R | R | R | S | R | R | R | | | |
| 89 | 4735 | 9 | M | PAED | UTI | URINE | PSEUDO. AEURO | S | R | S | R | R | R | S | S | S | S | | | |
| 90 | 4186 | 15 | F | SURGERY | WOUND INF | PUS | PSEUDO. AEURO | S | S | S | R | S | S | S | S | S | S | | | |
| 91 | 4183 | 72 | M | MEDICINE | PNEUMONIA | SPUTUM | PSEUDO. AEURO | S | R | S | R | S | R | S | S | S | S | | | |
| 92 | 4681 | 14 | M | URO | PYELO | URINE | PSEUDO. AEURO | S | R | S | R | R | R | R | S | S | S | N | N | N |
| 93 | 4229 | 26 | F | MEDICINE | PNEUMONIA | SPUTUM | PSEUDO. AEURO | S | S | R | R | S | S | S | S | S | S | | | |
| 94 | 5320 | 18 | F | ORTHO | WOUND INF | PUS | ACINETO | S | S | R | S | S | S | S | S | S | S | | | |
| 95 | 5344 | 30 | M | MEDICINE | PYLEO | URINE | PSEUDO. AEURO | S | S | S | R | S | S | S | S | S | S | | | |
| 96 | 5369 | 29 | M | MEDICINE | ABSCESS | PUS | PSEUDO. AEURO | S | S | S | R | R | S | S | S | S | S | | | |
| 97 | 5381 | 45 | M | MEDICINE | PNEUMONIA | SPUTUM | PSEUDO. AEURO | S | S | S | R | S | S | S | S | S | S | | | |
| 98 | 5385 | 60 | M | SURGERY | ABSCESS | PUS | PSEUDO. AEURO | S | S | S | R | S | S | S | S | S | S | | | |
| 99 | 5406 | 24 | M | ENT | SINUSITIS | PUS | ACINETO | S | S | R | R | S | R | S | S | S | S | | | |
| 100 | 5410 | 48 | F | MEDICINE | PNEUMONIA | SPUTUM | PSEUDO. AEURO | S | S | R | R | R | R | S | R | S | S | | | |

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